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(54) Title: METHOD TO PRODUCE VIRUS IN CULTURED CELLS SUPPLEMENTED WITH ALPHA-KETOGLUTARATE

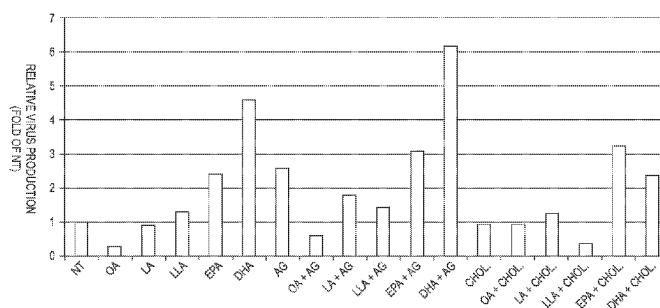


Figure 1

(57) **Abstract:** The present disclosure provides a method to enhance the production of virus in cultured fibroblasts by supplementing the cells with a TCA cycle intermediate, aketoglutarate, or a derivative thereof, wherein virus production is enhanced compared to the same method carried out in the absence of a-ketoglutarate or the derivative thereof. In view of the art, the method provides an unexpected improvement on methods routinely practiced.

# METHOD TO PRODUCE VIRUS IN CULTURED CELLS SUPPLEMENTED WITH ALPHA-KETOGLUTARATE

## STATEMENT OF GOVERNMENT INTEREST

[0001] This invention was made with government support under Grant Number CA85786, awarded by the National Institutes of Health (NIH), and Grant Number DP1DA026192 awarded by the NIH. The government has certain rights in the invention.

## FIELD OF THE INVENTION

[0002] The present disclosure relates to processes for virus production.

## BACKGROUND

[0003] Since the ability to obtain adequate viral yields can limit vaccine manufacturing, improved methods of virus production are always needed to meet an important industrial and medical need. Previous work (Munger et al., *PLoS Pathog* 2:e132, 2006; Munger et al., *Nat Biotech* 26:1179-86, 2008)) has demonstrated that human cytomegalovirus (HCMV) induces the synthesis of fatty acids, and, importantly, that the virus requires the *de novo* synthesis of fatty acids to generate an optimal yield of infectious progeny. Despite this understanding, US Patent No. 5,360,736 discloses that that addition of lipids during growth of certain viruses, and in particular after initiation of infection of the cultured cells, inhibits virus production.

[0004] Preparation of stock virus is necessary for development of therapeutic methods and materials. Accordingly, improved methods for virus production are useful for improving virus yield, and more specifically for vaccine production.

## DESCRIPTION OF THE DRAWINGS

[0005] Fig. 1. The effect of different fatty acids as medium supplement on HCMV yields.

[0006] Fig. 2. The effect of carbonyl/free radical scavenging compounds on HCMV yields.

[0007] Fig. 3. The effect of supplementing the cells with AA or DHA on VZV yields.

[0008] Fig. 4.  $\alpha$ T enhances the ability of AA and DHA to facilitate VZV replication.

[0009] Fig. 5. The effect of different processing methods on VZV yield.

[0010] Fig. 6. The effect of supplementing the cells with different combinations of fatty acids on VZV yield.

[0011] Fig. 7. Cholesterol enhances the ability of DHA to facilitate cell-free VZV production.

[0012] Fig. 8. The effect of DHA plus  $\alpha$ -T treatment on virus particle production and infectivity of VZV.

[0013] Fig. 9. The spread of VZV in the cells treated with DHA in combination with  $\alpha$ -T and cholesterol .

[0014] Fig. 10. The effect of supplementing the cells with  $\alpha$ -ketoglutarate on VZV yield.

[0015] Fig. 11. DHA,  $\alpha$ -tocopherol, and cholesterol supplementation cooperates with  $\alpha$ -ketoglutarate to enhance the production of cell-free VZV production

[0016] Fig. 12. Yield of VZV in glutamine-free medium

[0017] Fig 13. The effect of supplementing the cells with  $\alpha$ -ketoglutarate on HCMV yield.

### **DESCRIPTION OF THE INVENTION**

[0018] Provided herein is a method for increasing the yield of virus production from cultured cells. The present disclosure provides a method to enhance the production of virus in cultured fibroblasts by supplementing the cells with a TCA cycle intermediate,  $\alpha$ -ketoglutarate, or a derivative thereof, wherein virus production is enhanced compared to the same method carried out in the absence of  $\alpha$ -ketoglutarate or the derivative thereof. In view of the art, the method provides an unexpected improvement on methods routinely practiced. The method provided is useful in combination with routinely utilized variables relating to conditions of cell growth and cell maintenance, both prior to infection and after virus infection of the cells in culture, and in combination with known methods of harvesting, preparing, stabilizing and storing virus stocks, that are described in US Patent 5360736, incorporated herein in its entirety for all that it discloses, and/or known to those skilled in the art of virus propagation and preparation of virus stocks.

[0019] In various aspects,  $\alpha$ -ketoglutarate is utilized in its native form. In various aspects, a derivative of  $\alpha$ -ketoglutarate (dimethyl-  $\alpha$ -ketoglutarate,  $\alpha$ -kg, Willenborg et al., *Eur J Pharmacol*, **607** (1-3):41-6, 2009; Sigma) is utilized. Other derivatives of  $\alpha$ -ketoglutarate are well known in the art, and their use is also contemplated. For example,  $\alpha$ -ketoglutarate esters

are contemplated, including but not limited to octyl- $\alpha$ -ketoglutarate esters, benzyl- or 3-trifluoromethylbenzyl- $\alpha$ -ketoglutarate ester analogues as described in MacKenzie, et al., Mol Cell Biol. 2007 May; 27(9): 3282–3289 (the disclosure of which is incorporated herein in its entirety). Both cell-permeable and non-cell-permeable derivatives of  $\alpha$ -ketoglutarate are contemplated with the proviso that non-cell permeable derivatives are delivered to cells using delivery technology known in the art.

**[0020]** A method which utilizes  $\alpha$ -ketoglutarate and one or more derivative of  $\alpha$ -ketoglutarate are contemplated.

**[0021]** In a general embodiment, a method is provided for virus production wherein an infected host cell is cultured in the presence of  $\alpha$ -ketoglutarate, or a derivative thereof, in amount and for a time appropriate to allow virus production. The method provides increased virus production compared to the same method performed in the absence of  $\alpha$ -ketoglutarate, or the derivative thereof.

**[0022]** Accordingly, a method is provided for producing a virus comprising the step of culturing a host cell infected with a virus under conditions and for a time appropriate for producing the virus, wherein the conditions include  $\alpha$ -ketoglutarate, or a derivative thereof, in an amount and for a time effective to permit virus production. Production of virus is measured, in various aspects, by (i) the number of infectious virus particles, (ii) the number of virus particles, infectious and non-infectious, (iii) an amount of a specific viral antigen, and/or (iv) combinations of (i)-(iii). The method increases virus yield compared to the same method under conditions that do not include  $\alpha$ -ketoglutarate, or a derivative thereof.

**[0023]** In various aspects of the method, the conditions include the presence of  $\alpha$ -ketoglutarate, or a derivative thereof, and a fatty acid and/or cholesterol. In various aspects of the method, the conditions further include a scavenging compound. In various aspects, the method is carried out under conditions which include  $\alpha$ -ketoglutarate, or a derivative thereof, and no more than one fatty acid, no more than two fatty acids, no more than three fatty acids or no more than four fatty acids. In various aspects of the method, the conditions include  $\alpha$ -ketoglutarate, or a derivative thereof and at least two different fatty acids, at least three different fatty acids, at least four different fatty acids or four or more different fatty acids. In various aspects, the fatty acid or fatty acids is/are essentially homogeneous. An "essentially homogeneous" fatty is defined that includes about 5% or less contaminating fatty acids. For example and only for purposes of explanation, an essentially homogeneous fatty acid X

includes about 5% or less non-fatty acid Z (which can be one or more fatty acids), wherein non-fatty acid X is a fatty acid that is not fatty acid Z.

[0024] The method provided, in various aspects, further comprises the step of isolating said virus from medium of cell growth. In various aspects, the method further comprises the step of isolating the virus from the host cell. In various aspects, the method further comprises the step of infecting the host cells with the virus. In various aspects, the host cell is infected with a virus at different multiplicities of infection, at a multiplicity of infection (MOI) of between about 1:25 (i.e., 1 infected cell per 25 uninfected cells) and 1:625, of about 1:25, of about 1:125 or higher, or of between about 1:7 and 1:625. The method provided, in various aspects, further comprises the step of growing the host cells to confluence, to about 90% , about 80% confluence, about 70% confluence, about 60% confluence, about 50% confluence, or less than 50% confluence prior to infecting the host cells with the virus. The method in various aspects, further comprises the step of culturing the host cells after infecting the host cells with the virus. In various aspects, the method further comprises the step of adding or changing medium of growth for the host cells prior to isolating the virus. In various aspects, the method further comprises the step of incubating the host cells with an infecting virus for an adsorption period. In various aspects, the method further comprises the step of introducing  $\alpha$ -ketoglutarate, or a derivative thereof, with or without a fatty acid, cholesterol and/or scavenging compound during the step of adding or changing the medium. The method, in various aspects, further comprises the step of introducing  $\alpha$ -ketoglutarate, or a derivative thereof, with or without a fatty acid, cholesterol and/or scavenging compound prior to infecting the host cell with the virus, and/or introducing  $\alpha$ -ketoglutarate, or a derivative thereof, with or without a fatty acid, cholesterol and/or scavenging compound after infecting the host cell with the virus. In various aspects, the method further comprises the step of introducing  $\alpha$ -ketoglutarate, or a derivative thereof , with or without a fatty acid, cholesterol and/or scavenging compound at more than one time during the step of culturing the cells. An advantage of such repeated administration is the ability to maintain the desirable levels of the yield-enhancing components without reaching toxic levels at any point in the process, and the ability to tailor the levels of such yield-enhancing compounds to the specific demands of different stages of viral replication. In various aspects, the method further comprises the step of freezing the host cells prior to isolating the virus. In various aspects, the method further comprises the step isolating the virus without freezing the host cells. In various aspects, the method further comprises the step of sonicating the host cells to isolate the virus.

[0025] The method, in various aspects, further comprises the step of freezing the host cells prior to isolating the virus. In various aspects, the method further comprises the step of isolating the virus without freezing the host cells. In various aspects, method further comprises the step of sonicating the host cells to isolate the virus.

[0026] In various aspects, the method utilizes a host cell that is infection-susceptible to the virus, a host cell that is mammalian, a host cell is human, a host cell that is a fibroblast cell, or a host cell that is an MRC5 cell. In various aspects, the method utilizes a host cell that is an epithelial cell, a host cell that is a retinal cell, or a host cell that is an ARPE-19 cell. Those of ordinary skill in the art will readily appreciate that a large number of different cell types are amenable to use in the method and are contemplated by the disclosure.

[0027] In various aspects, the method is used with (and to produce) an enveloped DNA virus, a herpes virus, an alpha family herpes virus, a beta family herpes virus, a gamma family herpes virus, varicella zoster virus (VZV), cytomegalovirus (CMV), a pox virus, a non-enveloped picorna virus, including for example, but not limited to poliovirus, rhinovirus, hepatitis A virus, or foot and mouth disease virus, an RNA virus, influenza virus, herpes simplex virus, Epstein Barr virus, hepatitis C virus, Dengue virus, HIV, mumps virus, measles virus, rotavirus and/or parainfluenza virus.

[0028] In various aspects, the method utilizes cholesterol which is a cholesterol derivative or a cholesterol ester.

[0029] The method, in various aspects, utilizes a fatty acid which is a long chain fatty acid or a very long chain fatty acid, an omega-3 fatty acid, an omega-6 fatty acid, a naturally-occurring fatty acid, a derivative of a naturally-occurring fatty acid, a non-naturally-occurring fatty acid, a free fatty acid, a fatty acid ester, a fatty acid derivative, a triglyceride, a diglyceride, a monoglyceride, a phospholipid, a fatty acid that has at least 18 carbon, a fatty acid that has at least 20 carbons, a fatty acid that has at least 22 carbons, a fatty acid has at least 24 carbons, a fatty acid that has at least 26 carbon, a fatty acid that has at least 28 carbons, a fatty acid that has at least 30 carbons, a fatty acid has at least 32 carbons, a fatty acid that has at least 34 carbon, a fatty acid that has at least 36 carbons, a fatty acid that has at least 38 carbons, a fatty acid has at least 40 carbons, a fatty acid that is saturated, a fatty acid that is unsaturated, a fatty acid that is polyunsaturated, a fatty acid that has 1 or more double bonds, a fatty acid that has 2 or more double bonds, a fatty acid that has 3 and/or more double bonds, a fatty acid that has 4 or more double bonds, a fatty acid that has 5 or more double

bonds, a fatty acid that has 6 and/or more double bonds, a fatty acid that has 7 or more double bonds, a fatty acid that has 8 or more double bonds, a fatty acid that has 9 or more double bonds, a fatty acid that has 10 or more double bonds, a fatty acid that has 11 or more double bonds, or a fatty acid that has 12 or more double bonds. In various aspects, the fatty acid is selected from the group consisting of oleic acid (OA), linoleic acid (LA),  $\alpha$ -linolenic acid (LLA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), arachidonic acid (AA), hexacosanoic acid (HSA), octacosanoic acid (OSA),  $\alpha$ -linolenic acid and/or  $\gamma$ -linolenic acid.

**[0030]** In various aspects, method utilizes  $\alpha$ -ketoglutarate, or a derivative thereof, a fatty acid and/or cholesterol that is formulated in a mixture that improves delivery to and/or uptake in cells. In various aspects,  $\alpha$ -ketoglutarate, or a derivative thereof, the fatty acid and/or cholesterol is associated with a polymer. In various aspects,  $\alpha$ -ketoglutarate, or a derivative thereof, the fatty acid and/or cholesterol is associated with a protein and/or a synthetic polymer. In various aspects, the fatty acid and/or cholesterol is associated with a small molecule. In various aspects,  $\alpha$ -ketoglutarate, or a derivative thereof, the fatty acid and/or cholesterol is associated with cyclodextrin.

**[0031]** In various aspects, the method utilizes  $\alpha$ -ketoglutarate, or a derivative thereof and a scavenging compound that is a carbonyl scavenging compound and/or a free radical scavenging compound. The method, in various aspects, utilizes a carbonyl scavenging compound and a free radical scavenging compound. In various aspects, the method utilizes a scavenging compound that is selected from the group consisting of aminoguanidine,  $\alpha$ -tocopherol, hydralazine, glycosylisovitexin, N-acetyl-cystein, metformin, penicillamine, pyridoxamine, edaravone (EDA), tenilsetam, lipoic acid, 3,3-dimethyl-D-cysteine (DMC), L-3,3-dimethyl-D-cysteine (L-DMC), N-acetyl-3,3-dimethyl-D-cysteine (ADMC), N<sup>α</sup>-acetyl-L-cysteine (NAC), 3,3-dimethyl-D-cysteine-disulfide (DMCSS), S-methyl-DMC (SMDMC), L-cysteine (CYS), L-cysteine-O-methylester (CYSM), 3,3-dimethyl-D-cysteine-methylester (DMCM), 3-methyl-3-ethyl-D-cysteine (MEC), semicarbazide hydrochloride SC (hydrazine carboxamide), 1,1-dimethyl-biguanide hydrochloride (DMBG), N-tertbutylhydroxylamine (BHA), a flavonoid, a flavanol, epicatechin, a flavanone, naringenin, a flavonol, quercetin, a flavones, luteolin, an isoflavone, genistein, an anthocyanidin, cyanidin, a phenol/ phenolic acid, a flavan-3-ol compound, procyanidins B1 (9.8), procyanidins B2, (+)-catechin, (–)-epicatechin, caftaric acid, caffeic acid, and kaempferol.

**[0032]** In various aspects, the method utilizes  $\alpha$ -ketoglutarate, or a derivative thereof, at a concentration of greater than 1 mM, 1.1 mM, 1.2 mM, 1.3 mM, 1.4 mM, 1.5 mM, 1.6 mM,

1.7 mM, 1.8 mM, 1.9 mM, 2 mM, 2.1 mM, 2.2 mM, 2.3 mM, 2.4 mM, 2.5 mM, 2.6 mM, 2.7 mM, 2.8 mM, 2.9 mM, 3 mM, 3.1 mM, 3.2 mM, 3.3 mM, 3.4 mM, 3.5 mM, 3.6 mM, 3.7 mM, 3.8 mM, 3.9 mM, 4 mM, 4.1 mM, 4.2 mM, 4.3 mM, 4.4 mM, 4.5 mM, 4.6 mM, 4.7 mM, 4.8 mM, 4.9 mM, 5 mM, 5.1 mM, 5.2 mM, 5.3 mM, 5.4 mM, 5.5 mM, 5.6 mM, 5.7 mM, 5.8 mM, 5.9 mM, 6 mM, 6.1 mM, 6.2 mM, 6.3 mM, 6.4 mM, 6.5 mM, 6.6 mM, 6.7 mM, 6.8 mM, 6.9 mM, 7 mM, 7.1 mM, 7.2 mM, 7.3 mM, 7.4 mM, 7.5 mM, 7.6 mM, 7.7 mM, 7.8 mM, 7.9 mM, 8 mM, 8.1 mM, 8.2 mM, 8.3 mM, 8.4 mM, 8.5 mM, 8.6 mM, 8.7 mM, 8.8 mM, 8.9 mM, 9 mM, 9.1 mM, 9.2 mM, 9.3 mM, 9.4 mM, 9.5 mM, 9.6 mM, 9.7 mM, 9.8 mM, 9.9 mM, 10 mM, 10.1 mM, 10.2 mM, 10.3 mM, 10.4 mM, 10.5 mM, 10.6 mM, 10.7 mM, 10.8 mM, 10.9 mM, 11 mM, 11.1 mM, 11.2 mM, 11.3 mM, 11.4 mM, 11.5 mM, 11.6 mM, 11.7 mM, 11.8 mM, 11.9 mM, 12 mM, 12.1 mM, 12.2 mM, 12.3 mM, 12.4 mM, 12.5 mM, 12.6 mM, 12.7 mM, 12.8 mM, 12.9 mM, 13 mM, 13.1 mM, 13.2 mM, 13.3 mM, 13.4 mM, 13.5 mM, 13.6 mM, 13.7 mM, 13.8 mM, 13.9 mM, 14 mM, 14.1 mM, 14.2 mM, 14.3 mM, 14.4 mM, 14.5 mM, 14.6 mM, 14.7 mM, 14.8 mM, 14.9 mM, 15 mM, 15.1 mM, 15.2 mM, 15.3 mM, 15.4 mM, 15.5 mM, 15.6 mM, 15.7 mM, 15.8 mM, 15.9 mM, 16 mM, 16.1 mM, 16.2 mM, 16.3 mM, 16.4 mM, 16.5 mM, 16.6 mM, 16.7 mM, 16.8 mM, 16.9 mM, 17 mM, 17.1 mM, 17.2 mM, 17.3 mM, 17.4 mM, 17.5 mM, 17.6 mM, 17.7 mM, 17.8 mM, 17.9 mM, 18 mM, 18.1 mM, 18.2 mM, 18.3 mM, 18.4 mM, 18.5 mM, 18.6 mM, 18.7 mM, 18.8 mM, 18.9 mM, 19 mM, 19.1 mM, 19.2 mM, 19.3 mM, 19.4 mM, 19.5 mM, 19.6 mM, 19.7 mM, 19.8 mM, 19.9 mM, 20 mM, 20.1 mM, 20.2 mM, 20.3 mM, 20.4 mM, 20.5 mM, 20.6 mM, 20.7 mM, 20.8 mM, 20.9 mM, 21 mM, 21.1 mM, 21.2 mM, 21.3 mM, 21.4 mM, 21.5 mM, 21.6 mM, 21.7 mM, 21.8 mM, 21.9 mM, 22 mM, 22.1 mM, 22.2 mM, 22.3 mM, 22.4 mM, 22.5 mM, 22.6 mM, 22.7 mM, 22.8 mM, 22.9 mM, 23 mM, 23.1 mM, 23.2 mM, 23.3 mM, 23.4 mM, 23.5 mM, 23.6 mM, 23.7 mM, 23.8 mM, 23.9 mM, 24 mM, 24.1 mM, 24.2 mM, 24.3 mM, 24.4 mM, 24.5 mM, 24.6 mM, 24.7 mM, 24.8 mM, 24.9 mM, 25 mM, or more.

**[0033]** In various aspects, the method utilizes  $\alpha$ -ketoglutarate, or a derivative thereof, at a concentration up to 2 mM, 2.1 mM, 2.2 mM, 2.3 mM, 2.4 mM, 2.5 mM, 2.6 mM, 2.7 mM, 2.8 mM, 2.9 mM, 3 mM, 3.1 mM, 3.2 mM, 3.3 mM, 3.4 mM, 3.5 mM, 3.6 mM, 3.7 mM, 3.8 mM, 3.9 mM, 4 mM, 4.1 mM, 4.2 mM, 4.3 mM, 4.4 mM, 4.5 mM, 4.6 mM, 4.7 mM, 4.8 mM, 4.9 mM, 5 mM, 5.1 mM, 5.2 mM, 5.3 mM, 5.4 mM, 5.5 mM, 5.6 mM, 5.7 mM, 5.8 mM, 5.9 mM, 6 mM, 6.1 mM, 6.2 mM, 6.3 mM, 6.4 mM, 6.5 mM, 6.6 mM, 6.7 mM, 6.8 mM, 6.9 mM, 7 mM, 7.1 mM, 7.2 mM, 7.3 mM, 7.4 mM, 7.5 mM, 7.6 mM, 7.7 mM, 7.8

mM, 7.9 mM, 8 mM, 8.1 mM, 8.2 mM, 8.3 mM, 8.4 mM, 8.5 mM, 8.6 mM, 8.7 mM, 8.8 mM, 8.9 mM, 9 mM, 9.1 mM, 9.2 mM, 9.3 mM, 9.4 mM, 9.5 mM, 9.6 mM, 9.7 mM, 9.8 mM, 9.9 mM, 10 mM, 10.1 mM, 10.2 mM, 10.3 mM, 10.4 mM, 10.5 mM, 10.6 mM, 10.7 mM, 10.8 mM, 10.9 mM, 11 mM, 11.1 mM, 11.2 mM, 11.3 mM, 11.4 mM, 11.5 mM, 11.6 mM, 11.7 mM, 11.8 mM, 11.9 mM, 12 mM, 12.1 mM, 12.2 mM, 12.3 mM, 12.4 mM, 12.5 mM, 12.6 mM, 12.7 mM, 12.8 mM, 12.9 mM, 13 mM, 13.1 mM, 13.2 mM, 13.3 mM, 13.4 mM, 13.5 mM, 13.6 mM, 13.7 mM, 13.8 mM, 13.9 mM, 14 mM, 14.1 mM, 14.2 mM, 14.3 mM, 14.4 mM, 14.5 mM, 14.6 mM, 14.7 mM, 14.8 mM, 14.9 mM, 15 mM, 15.1 mM, 15.2 mM, 15.3 mM, 15.4 mM, 15.5 mM, 15.6 mM, 15.7 mM, 15.8 mM, 15.9 mM, 16 mM, 16.1 mM, 16.2 mM, 16.3 mM, 16.4 mM, 16.5 mM, 16.6 mM, 16.7 mM, 16.8 mM, 16.9 mM, 17 mM, 17.1 mM, 17.2 mM, 17.3 mM, 17.4 mM, 17.5 mM, 17.6 mM, 17.7 mM, 17.8 mM, 17.9 mM, 18 mM, 18.1 mM, 18.2 mM, 18.3 mM, 18.4 mM, 18.5 mM, 18.6 mM, 18.7 mM, 18.8 mM, 18.9 mM, 19 mM, 19.1 mM, 19.2 mM, 19.3 mM, 19.4 mM, 19.5 mM, 19.6 mM, 19.7 mM, 19.8 mM, 19.9 mM, 20 mM, 20.1 mM, 20.2 mM, 20.3 mM, 20.4 mM, 20.5 mM, 20.6 mM, 20.7 mM, 20.8 mM, 20.9 mM, 21 mM, 21.1 mM, 21.2 mM, 21.3 mM, 21.4 mM, 21.5 mM, 21.6 mM, 21.7 mM, 21.8 mM, 21.9 mM, 22 mM, 22.1 mM, 22.2 mM, 22.3 mM, 22.4 mM, 22.5 mM, 22.6 mM, 22.7 mM, 22.8 mM, 22.9 mM, 23 mM, 23.1 mM, 23.2 mM, 23.3 mM, 23.4 mM, 23.5 mM, 23.6 mM, 23.7 mM, 23.8 mM, 23.9 mM, 24 mM, 24.1 mM, 24.2 mM, 24.3 mM, 24.4 mM, 24.5 mM, 24.6 mM, 24.7 mM, 24.8 mM, 24.9 mM, 25 mM, or less.

**[0034]** In various aspects, the method utilizes  $\alpha$ -ketoglutarate, or a derivative thereof, at a concentration between 0.5 mM and 25 mM, 0.5 mM and 24 mM, 0.5 mM and 23 mM, 0.5 mM and 22 mM, 0.5 mM and 21 mM, 0.5 mM and 20 mM, 0.5 mM and 19 mM, 0.5 mM and 18 mM, 0.5 mM and 17 mM, 0.5 mM and 16 mM, 0.5 mM and 15 mM, 0.5 mM and 14 mM, 0.5 mM and 13 mM, 0.5 mM and 12 mM, 0.5 mM and 11 mM, 0.5 mM and 10 mM, 0.5 mM and 9 mM, 0.5 mM and 8 mM, 0.5 mM and 7 mM, 0.5 mM and 6 mM, or 0.5 mM and 5 mM. In various aspect, In various aspects, the method utilizes  $\alpha$ -ketoglutarate, or a derivative thereof, at a concentration between 0.5 mM and 25 mM, 1 mM and 25 mM, 2 mM and 25 mM, 3 mM and 25 mM, 4 mM and 25 mM, 5 mM and 25 mM, 6 mM and 25 mM, 7 mM and 25 mM, 8 mM and 25 mM, 9 mM and 25 mM, 10 mM and 25 mM, 11 mM and 25 mM, 12 mM and 25 mM, 13 mM and 25 mM, 14 mM and 25 mM, 15 mM and 25mM, 16 mM and 25 mM, 17 mM and 25 mM, 18 mM and 25 mM, 19 mM and 25mM, or 20 mM and 25 mM. In various aspects, the method utilizes  $\alpha$ -ketoglutarate, or a derivative thereof, at a concentration between 0.5 mM and 25 mM, 1 mM and 20 mM, 1 mM and 15 mM, 1 mM and 10 mM, 5

mM and 25 mM, 5 mM and 20 mM, 5 mM and 15 mM, 5 mM and 10 mM, 7.5 mM and 25 mM, 7.5 mM and 20 mM, 7.5 mM and 15 mM, or 7.5 mM and 10 mM.

**[0035]** In various aspects, the method utilizes a fatty acid that is present at a concentration of at least 5  $\mu$ M, at least 10  $\mu$ M, at least 15  $\mu$ M, at least 20  $\mu$ M, at least 25  $\mu$ M, at least 30  $\mu$ M, at least 35  $\mu$ M, at least 40  $\mu$ M, at least 45  $\mu$ M, at least 50  $\mu$ M, at least 55  $\mu$ M, at least 60  $\mu$ M, at least 65  $\mu$ M, at least 70  $\mu$ M, at least 75  $\mu$ M, at least 80  $\mu$ M, at least 85  $\mu$ M, at least 90  $\mu$ M, at least 95  $\mu$ M, at least 100  $\mu$ M, at least 110  $\mu$ M, at least 120  $\mu$ M, at least 130  $\mu$ M, at least 140  $\mu$ M, at least 150  $\mu$ M or more, and wherein the fatty acid is present at a concentration of 500  $\mu$ M or less, or at a concentration that is not toxic to the host cell.

Aspects of the methods include use of a fatty acid in a range of about 1  $\mu$ M to about 100  $\mu$ M, about 5  $\mu$ M to about 100  $\mu$ M, about 5  $\mu$ M to about 90  $\mu$ M, about 5  $\mu$ M to about 85, about 5  $\mu$ M to about 80  $\mu$ M, about 5  $\mu$ M to about 75  $\mu$ M, about 5  $\mu$ M to about 70  $\mu$ M, about 5  $\mu$ M to about 65  $\mu$ M, about 5  $\mu$ M to 60 about  $\mu$ M, about 5  $\mu$ M to about 55  $\mu$ M, or about 5  $\mu$ M to about 50  $\mu$ M. Aspects of the methods also include use of a fatty acid in a range of about 1  $\mu$ M to about 100  $\mu$ M, about 5  $\mu$ M to about 100  $\mu$ M, about 10  $\mu$ M to about 100  $\mu$ M, about 15  $\mu$ M to about 100  $\mu$ M, about 20  $\mu$ M to about 100  $\mu$ M, about 25  $\mu$ M to about 100  $\mu$ M, about 30  $\mu$ M to about 100  $\mu$ M, about 35  $\mu$ M to about 100  $\mu$ M, about 40  $\mu$ M to 100 about  $\mu$ M, about 45  $\mu$ M to about 100  $\mu$ M, or about 50  $\mu$ M to about 100  $\mu$ M. Aspects of the methods also include use of a fatty acid in a range of about 1  $\mu$ M to about 100  $\mu$ M, about 5  $\mu$ M to about 95  $\mu$ M, about 10  $\mu$ M to about 90  $\mu$ M, about 15  $\mu$ M to about 85  $\mu$ M, about 20  $\mu$ M to about 80  $\mu$ M, about 25  $\mu$ M to about 75  $\mu$ M, about 30  $\mu$ M to about 70  $\mu$ M, about 35  $\mu$ M to about 65  $\mu$ M, about 40  $\mu$ M to 60 about  $\mu$ M, or about 45  $\mu$ M to about 55  $\mu$ M.

**[0036]** In various aspects, the method utilizes cholesterol that is present at a concentration of at least 5  $\mu$ M, at least 10  $\mu$ M, at least 15  $\mu$ M, at least 20  $\mu$ M, at least 25  $\mu$ M, at least 30  $\mu$ M, at least 35  $\mu$ M, at least 40  $\mu$ M, at least 45  $\mu$ M, at least 50  $\mu$ M, at least 55  $\mu$ M, at least 60  $\mu$ M, at least 65  $\mu$ M, at least 70  $\mu$ M, at least 75  $\mu$ M, at least 80  $\mu$ M, at least 85  $\mu$ M, at least 90  $\mu$ M, at least 95  $\mu$ M, at least 100  $\mu$ M, at least 110  $\mu$ M, at least 120  $\mu$ M, at least 130  $\mu$ M, at least 140  $\mu$ M, at least 150  $\mu$ M or more and wherein cholesterol is present at a concentration of 500  $\mu$ M or less, or at a concentration that is not toxic to the host cell. In various aspects, the cholesterol is present at a concentration of less than 450  $\mu$ M, 400  $\mu$ M, 350  $\mu$ M 300  $\mu$ M, 250  $\mu$ M, 200  $\mu$ M or 150  $\mu$ M. In various aspects, the cholesterol is present at a concentration of less than 450  $\mu$ M, 400  $\mu$ M, 350  $\mu$ M 300  $\mu$ M, 250  $\mu$ M, 200  $\mu$ M or 150  $\mu$ M. Aspects of the methods include use of cholesterol in a range of about 1  $\mu$ M to about 100  $\mu$ M, about 5  $\mu$ M to

about 100  $\mu\text{M}$ , about 5  $\mu\text{M}$  to about 90  $\mu\text{M}$ , about 5  $\mu\text{M}$  to about 85, about 5  $\mu\text{M}$  to about 80  $\mu\text{M}$ , about 5  $\mu\text{M}$  to about 75  $\mu\text{M}$ , about 5  $\mu\text{M}$  to about 70  $\mu\text{M}$ , about 5  $\mu\text{M}$  to about 65  $\mu\text{M}$ , about 5  $\mu\text{M}$  to 60 about  $\mu\text{M}$ , about 5  $\mu\text{M}$  to about 55  $\mu\text{M}$ , or about 5  $\mu\text{M}$  to about 50  $\mu\text{M}$ .

Aspects of the methods also include use of cholesterol in a range of about 1  $\mu\text{M}$  to about 100  $\mu\text{M}$ , about 5  $\mu\text{M}$  to about 100  $\mu\text{M}$ , about 10  $\mu\text{M}$  to about 100  $\mu\text{M}$ , about 15  $\mu\text{M}$  to about 100  $\mu\text{M}$ , about 20  $\mu\text{M}$  to about 100  $\mu\text{M}$ , about 25  $\mu\text{M}$  to about 100  $\mu\text{M}$ , about 30  $\mu\text{M}$  to about 100  $\mu\text{M}$ , about 35  $\mu\text{M}$  to about 100  $\mu\text{M}$ , about 40  $\mu\text{M}$  to 100 about  $\mu\text{M}$ , about 45  $\mu\text{M}$  to about 100  $\mu\text{M}$ , or about 50  $\mu\text{M}$  to about 100  $\mu\text{M}$ . Aspects of the methods also include use of cholesterol in a range of about 1  $\mu\text{M}$  to about 100  $\mu\text{M}$ , about 5  $\mu\text{M}$  to about 95  $\mu\text{M}$ , about 10  $\mu\text{M}$  to about 90  $\mu\text{M}$ , about 15  $\mu\text{M}$  to about 85  $\mu\text{M}$ , about 20  $\mu\text{M}$  to about 80  $\mu\text{M}$ , about 25  $\mu\text{M}$  to about 75  $\mu\text{M}$ , about 30  $\mu\text{M}$  to about 70  $\mu\text{M}$ , about 35  $\mu\text{M}$  to about 65  $\mu\text{M}$ , about 40  $\mu\text{M}$  to 60 about  $\mu\text{M}$ , or about 45  $\mu\text{M}$  to about 55  $\mu\text{M}$ .

[0037] In various aspects, the method utilizes a scavenging compound that is present at a concentration at least 5  $\mu\text{M}$ , at least 10  $\mu\text{M}$ , at least 15  $\mu\text{M}$ , at least 20  $\mu\text{M}$ , at least 25 $\mu\text{M}$ , at least 30  $\mu\text{M}$ , at least 35  $\mu\text{M}$ , at least 40  $\mu\text{M}$ , at least 45  $\mu\text{M}$ , at least 50  $\mu\text{M}$ , at least 55  $\mu\text{M}$ , at least 60  $\mu\text{M}$ , at least 65  $\mu\text{M}$ , at least 70  $\mu\text{M}$ , at least 75  $\mu\text{M}$ , at least 80  $\mu\text{M}$ , at least 85 $\mu\text{M}$ , at least 90  $\mu\text{M}$ , at least 95  $\mu\text{M}$ , at least 100  $\mu\text{M}$ , at least 110  $\mu\text{M}$ , at least 120  $\mu\text{M}$ , at least 130  $\mu\text{M}$ , at least 140  $\mu\text{M}$ , at least 150  $\mu\text{M}$  or more, and wherein the scavenging compound is present at a concentration of 500  $\mu\text{M}$  or less, or at a concentration that is not toxic to the host cell. In various aspects, the scavenger compound is present at a concentration of less than 450  $\mu\text{M}$ , 400  $\mu\text{M}$ , 350  $\mu\text{M}$  300  $\mu\text{M}$ , 250  $\mu\text{M}$ , 200  $\mu\text{M}$  or 150  $\mu\text{M}$ . Aspects of the method include use of a scavenger compound in a range of about 1  $\mu\text{M}$  to about 10 mM, about 1  $\mu\text{M}$  to about 9 mM, about 1  $\mu\text{M}$  to about 8 mM, about 1  $\mu\text{M}$  to about 7 mM, about 1  $\mu\text{M}$  to about 6 mM, about 1  $\mu\text{M}$  to about 5 mM, about 1  $\mu\text{M}$  to about 4 mM, about 1  $\mu\text{M}$  to about 3 mM, about 1  $\mu\text{M}$  to about 2 mM, about 1  $\mu\text{M}$  to about 1 mM, about 1  $\mu\text{M}$  to about 950  $\mu\text{M}$ , about 1  $\mu\text{M}$  to about 900  $\mu\text{M}$ , about 1  $\mu\text{M}$  to about 850  $\mu\text{M}$ , about 1  $\mu\text{M}$  to about 800  $\mu\text{M}$ , about 1  $\mu\text{M}$  to about 750  $\mu\text{M}$ , about 1  $\mu\text{M}$  to about 700  $\mu\text{M}$ , about 1  $\mu\text{M}$  to about 650  $\mu\text{M}$ , about 1  $\mu\text{M}$  to about 600  $\mu\text{M}$ , about 1  $\mu\text{M}$  to about 550  $\mu\text{M}$ , about 1  $\mu\text{M}$  to about 500  $\mu\text{M}$ , about 1  $\mu\text{M}$  to about 450  $\mu\text{M}$ , about 1  $\mu\text{M}$  to about 400  $\mu\text{M}$ , about 1  $\mu\text{M}$  to about 350  $\mu\text{M}$ , about 1  $\mu\text{M}$  to about 300  $\mu\text{M}$ , about 1  $\mu\text{M}$  to about 250  $\mu\text{M}$ , about 1  $\mu\text{M}$  to about 200  $\mu\text{M}$  about 1  $\mu\text{M}$  to about 150  $\mu\text{M}$ , about 1  $\mu\text{M}$  to about 100  $\mu\text{M}$ , about 1  $\mu\text{M}$  to about 95  $\mu\text{M}$ , about 1  $\mu\text{M}$  to about 90  $\mu\text{M}$ , about 1  $\mu\text{M}$  to about 85  $\mu\text{M}$ , about 1  $\mu\text{M}$  to about 80  $\mu\text{M}$ , about 1  $\mu\text{M}$  to about 75  $\mu\text{M}$ , about 1  $\mu\text{M}$  to about 70  $\mu\text{M}$ , about 1  $\mu\text{M}$  to about 65  $\mu\text{M}$ , about 1  $\mu\text{M}$  to about

60  $\mu\text{M}$ , about 1  $\mu\text{M}$  to about 55  $\mu\text{M}$ , about 1  $\mu\text{M}$  to about 50  $\mu\text{M}$ , about 1  $\mu\text{M}$  to about 45  $\mu\text{M}$ , about 1  $\mu\text{M}$  to about 40  $\mu\text{M}$ , about 1  $\mu\text{M}$  to about 35  $\mu\text{M}$ , about 1  $\mu\text{M}$  to about 30  $\mu\text{M}$ , about 1  $\mu\text{M}$  to about 25  $\mu\text{M}$ , about 1  $\mu\text{M}$  to about 20  $\mu\text{M}$ , about 1  $\mu\text{M}$  to about 15  $\mu\text{M}$ , or about 1  $\mu\text{M}$  to about 10  $\mu\text{M}$ . Aspects of the method also include use of a scavenger compound in a range of about 1  $\mu\text{M}$  to about 10 mM, about 10  $\mu\text{M}$  to about 10 mM, about 20  $\mu\text{M}$  to about 10 mM, about 30  $\mu\text{M}$  to about 10 mM, about 40  $\mu\text{M}$  to about 10 mM, about 50  $\mu\text{M}$  to about 10 mM, about 60  $\mu\text{M}$  to about 10 mM, about 70  $\mu\text{M}$  to about 10 mM, about 80  $\mu\text{M}$  to about 10 mM, about 90  $\mu\text{M}$  to about 10 mM, about 100  $\mu\text{M}$  to about 10 mM, about 150  $\mu\text{M}$  to about 10 mM, about 200  $\mu\text{M}$  to about 10 mM, about 250  $\mu\text{M}$  to about 10 mM, about 300  $\mu\text{M}$  to about 10 mM, about 350  $\mu\text{M}$  to about 10 mM, about 400  $\mu\text{M}$  to about 10 mM, about 450  $\mu\text{M}$  to about 10 mM, about 500  $\mu\text{M}$  to about 10 mM, about 550  $\mu\text{M}$  to about 10 mM, about 600  $\mu\text{M}$  to about 10 mM, about 650  $\mu\text{M}$  to about 10 mM, about 700  $\mu\text{M}$  to about 10 mM, about 750  $\mu\text{M}$  to about 10 mM, about 800  $\mu\text{M}$  to about 10 mM, about 850  $\mu\text{M}$  to about 10 mM, about 900  $\mu\text{M}$  to about 10 mM, about 1 mM to about 10 mM, about 2 mM to about 10 mM, about 3 mM to about 10 mM, about 4 mM to about 10 mM, about 5 mM to about 10 mM, about 6 mM to about 10 mM, about 8 mM to about 10 mM, or about 9 mM to about 10 mM. Aspects of the method also include use of a scavenger compound in a range of about 1  $\mu\text{M}$  to about 10 mM, about 10  $\mu\text{M}$  to about 1 mM, about 50  $\mu\text{M}$  to about 950  $\mu\text{M}$ , about 100  $\mu\text{M}$  to about 900  $\mu\text{M}$ , about 150  $\mu\text{M}$  to about 850  $\mu\text{M}$ , about 200  $\mu\text{M}$  to about 800  $\mu\text{M}$ , about 250  $\mu\text{M}$  to about 750  $\mu\text{M}$ , about 300  $\mu\text{M}$  to about 700  $\mu\text{M}$ , about 350  $\mu\text{M}$  to about 650  $\mu\text{M}$ , about 400  $\mu\text{M}$  to about 600  $\mu\text{M}$ , about 450  $\mu\text{M}$  to about 550  $\mu\text{M}$ , or about 400  $\mu\text{M}$  to about 500  $\mu\text{M}$ .

**[0038]** In various aspects, the method utilizes a fatty acid that is present at a concentration of no more than 5  $\mu\text{M}$ , no more than 10  $\mu\text{M}$ , no more than 15  $\mu\text{M}$ , no more than 20  $\mu\text{M}$ , no more than 25  $\mu\text{M}$ , no more than 30  $\mu\text{M}$ , no more than 35  $\mu\text{M}$ , no more than 40  $\mu\text{M}$ , no more than 45  $\mu\text{M}$ , no more than 50  $\mu\text{M}$ , no more than 55  $\mu\text{M}$ , no more than 60  $\mu\text{M}$ , no more than 65  $\mu\text{M}$ , no more than 70  $\mu\text{M}$ , no more than 75  $\mu\text{M}$ , no more than 80  $\mu\text{M}$ , no more than 85  $\mu\text{M}$ , no more than 90  $\mu\text{M}$ , no more than 95  $\mu\text{M}$ , no more than 100  $\mu\text{M}$ , no more than 110  $\mu\text{M}$ , no more than 120  $\mu\text{M}$ , no more than 130  $\mu\text{M}$ , no more than 140  $\mu\text{M}$ , no more than 150  $\mu\text{M}$ .

**[0039]** In various aspects, the method utilizes cholesterol that is present at a concentration of no more than 5  $\mu\text{M}$ , no more than 10  $\mu\text{M}$ , no more than 15  $\mu\text{M}$ , no more than 20  $\mu\text{M}$ , no more than 25  $\mu\text{M}$ , no more than 30  $\mu\text{M}$ , no more than 35  $\mu\text{M}$ , no more than 40  $\mu\text{M}$ , no more

than 45  $\mu\text{M}$ , no more than 50  $\mu\text{M}$ , no more than 55  $\mu\text{M}$ , no more than 60  $\mu\text{M}$ , no more than 65  $\mu\text{M}$ , no more than 70  $\mu\text{M}$ , no more than 75  $\mu\text{M}$ , no more than 80  $\mu\text{M}$ , no more than 85  $\mu\text{M}$ , no more than 90  $\mu\text{M}$ , no more than 95  $\mu\text{M}$ , no more than 100  $\mu\text{M}$ , no more than 110  $\mu\text{M}$ , no more than 120  $\mu\text{M}$ , no more than 130  $\mu\text{M}$ , no more than 140  $\mu\text{M}$ , no more than 150  $\mu\text{M}$ .

[0040] In various aspects, the method utilizes a scavenging compound that is present at a concentration of no more than 5  $\mu\text{M}$ , no more than 10  $\mu\text{M}$ , no more than 15  $\mu\text{M}$ , no more than 20  $\mu\text{M}$ , no more than 25  $\mu\text{M}$ , no more than 30  $\mu\text{M}$ , no more than 35  $\mu\text{M}$ , no more than 40  $\mu\text{M}$ , no more than 45  $\mu\text{M}$ , no more than 50  $\mu\text{M}$ , no more than 55  $\mu\text{M}$ , no more than 60  $\mu\text{M}$ , no more than 65  $\mu\text{M}$ , no more than 70  $\mu\text{M}$ , no more than 75  $\mu\text{M}$ , no more than 80  $\mu\text{M}$ , no more than 85  $\mu\text{M}$ , no more than 90  $\mu\text{M}$ , no more than 95  $\mu\text{M}$ , no more than 100  $\mu\text{M}$ , no more than 110  $\mu\text{M}$ , no more than 120  $\mu\text{M}$ , no more than 130  $\mu\text{M}$ , no more than 140  $\mu\text{M}$ , no more than 150  $\mu\text{M}$ .

[0041] Additional aspects and details of the invention will be apparent from the following examples, which are intended to be illustrative rather than limiting.

## **EXAMPLES**

### **EXAMPLE 1**

[0042] The possibility that the yield of HCMV could be improved was tested by adding specific fatty acids to the medium of infected human MRC5 fibroblasts (American Type Culture Collection).

[0043] Cells were infected with the AD169 strain of HCMV at a multiplicity of 0.5 infectious units/cell, and immediately following a 2-hour adsorption period, cells were fed with medium (Dulbecco's Modified Eagle Medium, DMEM) containing 10% fetal calf serum plus various fatty acids, cholesterol and carbonyl scavenging compound. At 96 hours post infection, infectious virus in the medium was assayed by fluorescent focus assay using antibody to the HCMV IE1 protein.

[0044] Briefly, About 90% confluent MRC5 human fibroblasts were infected with HCMV at a multiplicity of 0.5 IU/cell. Two hours after infection, medium was replaced with fresh medium containing 10% fetal calf serum and either of oleic acid (OA, up to about 100  $\mu\text{M}$ ), linoleic acid (LA, up to about 100  $\mu\text{M}$ ),  $\alpha$ -linolenic acid (LLA, up to about 100  $\mu\text{M}$ ), eicosapentaenoic acid (EPA, up to about 75  $\mu\text{M}$ ), or docosahexaenoic acid (DHA, up to about

50  $\mu\text{M}$ ). The experiment was also performed in the presence of either aminoguanidine (AG, up to about 250  $\mu\text{M}$ ) or cholesterol (chol., up to about 13  $\mu\text{M}$ ). Virus production at 96 hours after infection was determined by fluorescent focus assay in MRC-5 cells and shown as a fold change relative to no treatment (NT) which was  $5 \times 10^5$  infectious units. The fold-changes are the average of two independent infections. Results are shown in Figure 1.

**[0045]** As is evident in Figure 1, oleic acid (OA) reduced the yield of HCMV; linoleic acid (LA) had little effect on the yield; and  $\alpha$ -linolenic acid (LLA) increased the yield by about 1.2-fold. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) increased HCMV yield by factors of 2.5 and 4.6, respectively. Further, although aminoguanidine alone increased the yield of HCMV by a factor of about 2.6, the increase resulting from addition of the carbonyl scavenging compound was reduced by inclusion of OA, LA or LLA. In contrast, aminoguanidine plus EPA gave a slightly higher yield than either additive alone, and the combination of aminoguanidine plus DHA increased the yield by a factor of 6.2, a substantially higher yield than achieved with no additive or either additive alone. Addition of cholesterol alone (cholesterol solution, Sigma Aldrich #S5442) had no effect on HCMV yield and it did not improve, and in some cases inhibited, the enhancing effects of fatty acids. These results show that the addition of fatty acids can enhance the yield of HCMV obtained from cultured MRC5 fibroblasts, and this enhancement can be further increased by inclusion of a carbonyl scavenging compound.

## EXAMPLE 2

**[0046]** Experiments along the line of those conducted in Example 1 were designed to determine whether the addition of other fatty acids or fatty acid derivatives, (e.g., arachidonic acid (AA) or its derivatives) alone or in combination with cholesterol or cholesterol derivatives, with or without aminoguanidine or another carbonyl scavenging compound or a free radical scavenging compound, could enhance the yield of HCMV.

**[0047]** Briefly, about 90% confluent MRC5 fibroblasts were infected with HCMV at a multiplicity of 0.5 IU/cell. Two hours after infection, medium was replaced with fresh medium containing 10% fetal calf serum and  $\alpha$ -tocopherol ( $\alpha$ -T) or aminoguanidine (AG) at indicated concentrations. Virus production at 96 h after infection was determined by fluorescent focus assay in which MRC-5 cells and shown as a fold change relative to no treatment (NT). The fold-changes are the average of two independent infections. Results are set out in Figure 2.

[0048] This enhancement could be observed in MRC5 fibroblasts, other fibroblasts or other cell types suitable for the growth of HCMV. To the extent that the alternative fatty acid, cholesterol, carbonyl scavenging compounds and cell types enhance the production of HCMV, this invention encompasses their use in the process of virus growth. Figure 2 shows an example of second carbonyl scavenging compound/free radical scavenging compound, alpha-tocopherol ( $\alpha$ T), which enhances the production of HCMV as observed for aminoguanidine.

[0049] Further, certain formulations of natural or artificial fatty acids, which can be elongated and/or unsaturated within cells to produce AA or DHA, respectively, are used to substitute for AA or DHA.

[0050] An exemplary, but not limiting, embodiment of this invention includes supplementation of medium supporting MRC5 cells with docosahexaenoic acid (DHA), a dietary-essential omega-3 polyunsaturated fatty acid (PUFA), plus aminoguanidine, a carbonyl scavenging compound.

### EXAMPLE 3

[0051] The possibility was tested that the yield of VZV also could be improved by adding specific fatty acids to the medium of infected human MRC5 fibroblasts.

[0052] For this test, MRC5 cells (passage 20 – 25) were seeded at a density of 300,000 cell/100 mm culture dish and grown in 15 ml of DMEM containing 10% fetal calf serum plus 2mM GlutaMAX (GIBCO®, GlutaMAX<sup>TM</sup>) at 35°C. A lipid mixture (LM-1, 1 ml/liter medium, Sigma Aldrich # L5146) was added to the cells either at the time of seeding or 1 day after seeding. Three days later, the culture medium was replaced with 10 ml growth medium containing 50 mM sucrose as a stabilizer. The cells were further incubated for 3 days and growth medium was replaced with fresh medium containing no sucrose. After cells reached confluence, they were infected with VZV by adding infected cells (1 infected cell/50 uninfected cells; infected cells were from a preparation frozen in a solution of 10% DMSO plus 90% fetal calf serum and stored in liquid nitrogen). At the time of infection, the cultures were re-fed with DMEM containing 10% fetal calf serum plus 2mM glutamax. Arachidonic acid (AA) + alpha-tocopherol ( $\alpha$ T) or DHA +  $\alpha$ T were added at the indicated times. 72 hours after infection, cells were washed twice with PBS, and incubated in 10 ml of PBS containing 50 mM ammonium chloride for 50 minutes at 4°C. The cells were harvested and frozen in PSGC buffer (Harper et al., *Arch Virol* 143:1163-70, 1998) at -80°C. Infectious virus was

subsequently quantified by plaque assay of sonicated cells on ARPE-19 cells (American Type Culture Collection). Results are set out in Figure 3.

[0053] Results indicates that addition of LM-1 during cell growth prior to infection enhanced the virus yield by a factor of nearly two, but addition at 1 day after infection did not enhance virus production. However, addition of AA +  $\alpha$ T or DHA +  $\alpha$ T at various times after infection enhanced the production of infectious virus, with the greatest enhancement of virus yield occurring when the fatty acid and carbonyl scavenging compound were added between 1-6 hours post infection.

[0054] The experiment was repeated, varying the amount of fatty acid and  $\alpha$ T added to MRC5 cells at 6 hours post infection and the results are set out in Figure 4.

[0055] Briefly, in these repeat experiments, MRC5 cells were infected with VZV at an MOI=1:50. AA, DHA, and  $\alpha$ T were added to the cells at 6 hpi as indicated. 72 hours after infection, the cells were harvested into PSGC buffer and frozen at -80°C for later processing. After thawing, the cells were sonicated and the yield of cell free VZV quantified by standard plaque assay on ARPE-19 cells. Fold change relative to no treatment (NT) is shown. (\*) indicates that the composition produced cytotoxicity that was evident upon visual inspection. The fold-changes are the average of two independent infections.

[0056] As shown in figure 4, in the absence of the carbonyl scavenging agent, 25  $\mu$ M AA enhanced the yield of virus, whereas 100  $\mu$ M AA inhibited virus production; in contrast, in the presence of  $\alpha$ T, both doses of AA increased the virus yield, with 100  $\mu$ M showing the greatest increase at 5 fold. Similarly, 25  $\mu$ M DHA alone increased the yield by a factor of about 1.5, whereas 25  $\mu$ M DHA +  $\alpha$ T produced a 7.5-fold increase. 100  $\mu$ M DHA was toxic in the absence or presence of  $\alpha$ T.

[0057] These experiments demonstrate that the addition of certain fatty acids together with a carbonyl scavenging agent after infection with VZV augment the production of infectious progeny. The addition of the non-essential fatty acid, oleic acid (100  $\mu$ M), reduced VZV production by a factor of 2, without causing observable cellular toxicity.

#### EXAMPLE 4

[0058] In the experiments presented in Figures 3 and 4, the infected cells were harvested into PSGC buffer, frozen, subsequently thawed and disrupted by sonication and then titered. Next the yield of infectious virus obtained by this method was compared to an alternative

method where infected cells were harvested into PSGC buffer, immediately disrupted by sonication, and then frozen at -80°C prior to titration.

[0059] In brief, MRC5 cells were infected with VZV at an MOI=1:50. Lipid mixture 1(LM-1) was added to the cells immediately after cell seeding. At 6 hours after infection, up to about 100  $\mu$ M AA or 25 up to about  $\mu$ M DHA was added to cells together with up to about 10  $\mu$ M  $\alpha$ T. 72 hours after infection, the cells were harvested into PSGC buffer and either frozen at -80°C and sonicated later for the release of virus (frozen cells) or immediately sonicated after harvesting and supernatants containing the cell-free VZV were frozen at -80°C (frozen sup.) prior to titration. Cell-free VZV yield was quantified by plaque assay on APRE-19 cells. Fold change relative to no treatment (NT) is shown. The numbers above the bars indicate the amount of virus obtained per ml in the corresponding treatment. The results are set out in Figure 5.

## EXAMPLE 5

[0060] Having improved the yield of infectious VZV by sonicating infected cells in PSGC buffer before freezing, tested the effect of additional fatty acids (hexacosanoic acid (HSA), and octacosanoic acid (OSA) and fatty acid combinations on virus production was tested. Results are set out in Figure 6.

[0061] Although HSA and OSA improved virus yields in comparison to no treatment, these additional fatty acids and combinations did not perform as well as DHA +  $\alpha$ T. Further, high doses of two combinations generated less virus than no treatment, presumably due to toxicity resulting from high total concentrations of the combined fatty acids.

[0062] Briefly, MRC5 cells were infected with VZV at an MOI=1:50. Six hours after infection cells were treated with indicated combinations of lipids plus 10  $\mu$ M  $\alpha$ T. Hexacosanoic acid (HSA) and octacosanoic acid (OSA) were dissolved in 20 mg/ml  $\alpha$ -cyclodextrin (Sigma-Aldrich) in PBS by sonication and added to a solution of 10 mg/ml fatty acid-free BSA (Sigma-Aldrich) in PBS (1:1, v/v) to give a stock concentration of 10 mM for each fatty acid. HSA, OSA, and DHA were used at 25  $\mu$ M, and AA was used at 25  $\mu$ M. Two sets of fatty acid concentrations was used for combination treatments: DHA, AA, and HSA was either added at concentrations of 25  $\mu$ M, 100  $\mu$ M, and 25  $\mu$ M (high), or 10  $\mu$ M, 50  $\mu$ M, and 10  $\mu$ M (low), respectively. 72 hours after infection, the cells were harvested into PSGC buffer, sonicated immediately and the yield of cell free VZV quantified by plaque assay on

ARPE-19 cells. Fold change relative to no treatment (NT) is shown. The fold-changes are the average of two independent infections. Results are shown in Figure 6.

[0063] It is possible that the relatively poor performance of HSA and OSA in the experiment presented in Figure 5 resulted from difficulty in achieving efficient delivery of the fatty acids to cells. Alternative formulations of the fatty acids are contemplated to improve uptake and stimulate more efficient virus production.

## EXAMPLE 6

[0064] Next the possibility that the addition of cholesterol would further enhance the elevated yields obtained by supplementation with fatty acids was tested.

[0065] MRC5 cells were infected at a MOI of 1:100, and harvested either at 48 or 72 hours after infection. As controls, the cells were treated with two different mixtures of lipids immediately after cell seeding. LM-1 is rich in omega-3 fatty acids, and LM-2 (Invitrogen, # 11905) is a chemically defined mixture that contains mainly omega-6 fatty acids.

[0066] Briefly, MRC5 cells were grown in DMEM containing 10 % fetal calf serum, 2 mM GlutaMAX) at 35°C as described in the text. Lipid mixture 1 (LM-1, Sigma) or 2 (LM-2, Invitrogen) was added to the cells immediately after seeding. The cells were infected with VZV at an MOI=1:100. Six hours after infection cells were treated with indicated lipid combinations plus 10  $\mu$ M  $\alpha$ T. HSA and DHA were used at 25  $\mu$ M, and AA was used at 100  $\mu$ M. Where indicated, 13  $\mu$ M cholesterol was added on the cells. 48 or 72 hours after infection, the cells were harvested into PSGC buffer, sonicated immediately and the yield of cell free VZV quantitated by standard plaque assay on ARPE-19 cells. Fold change relative to no treatment (NT) harvested at 48 hpi is shown. The numbers above the bars indicate the amount of virus obtained per ml in the corresponding treatment. The fold-changes are the average of two independent infections. Results are set out in Figure 7.

[0067] Both lipid mixtures slightly and similarly elevated VZV yields at both times. The effects of these lipid mixtures were not as large as the effects of the individual fatty acids. DHA, AA and HSA were tested with  $\alpha$ -T, and, as in previous experiments, each of these additives elevated the yield of VZV at 72 hours post infection. Cholesterol was also tested as a supplement and at 72 hr after infection, it increased the yield of VZV by a factor of about two relative to no treatment. Yields were much lower at 48 than at 72 hours after infection. Finally, the effect of cholesterol addition to DHA +  $\alpha$ -T and DHA + HSA +  $\alpha$ -T was tested,

and it proved to further increase the yield of VZV. At 72 hours post infection,  $9.6 \times 10^5$  PFU/ml of infectious VZV was achieved by supplementation with DHA +  $\alpha$ -T plus cholesterol.

## EXAMPLE 7

[0068] The yield of virus particles by quantifying the amount of viral DNA in virus stocks by using quantitative PCR (qPCR) was then quantified.

[0069] Virus stocks were treated with DNase I before qPCR analysis. Before DNase I treatment, cellular DNA was detected in virus stocks using primers specific for the actin locus, but after treatment with the enzyme, cellular DNA was no longer detected. This observation demonstrated that the DNase I treatment effectively degraded DNA in the virus stocks that was not protected within virus particles. Each copy of DNase I-resistant VZV DNA was taken as a proxy for one virus particle.

[0070] Briefly, cell-free VZV was obtained from the cells treated with the indicated combinations of lipid mixture (LM-1, Sigma), DHA (about 25  $\mu$ M) plus  $\alpha$ T (about 10  $\mu$ M), and cholesterol (about 13  $\mu$ M), as described in the legend to figure 7. The samples were treated with DNase I (2 units, 30 min, 37°C) to remove contaminating DNA outside the viral envelope and the number of particles containing viral genome was determined by quantitative real-time PCR analysis. In parallel, the amount of virus produced was determined by plaque assay and infectivity of the viruses was calculated by dividing the number of enveloped virus particles by number of infectious virus produced (particle/PFU). The results are shown as fold change relative to no treatment (NT).

[0071] The amount of infectivity in each sample was determined in parallel by plaque assay. As shown in Figure 8, the number of virus particles and the specific infectivity of the particles were little changed by LM-1 as compared to no treatment. Addition of DHA +  $\alpha$ T at 6 hours post infection increased the number of virus particles and also increased the particle/PFU ratio by a factor of nearly 2. Addition of DHA +  $\alpha$ T + cholesterol had no effect on the specific infectivity of virus particles (particles/PFU), but it increased the number of virus particles by a factor of 9.

[0072] Importantly, then, addition of DHA +  $\alpha$ T + cholesterol at 6 hours post infection increased the yield of virus particles and infectivity by a factor of 9 at 72 hours post infection as compared to no treatment.

**EXAMPLE 8**

[0073] Viral spread was monitored by assaying the size of infected foci at 72 hours post infection (Fig. 9).

[0074] Briefly, ARPE-19 and MRC5 cells were infected with VZV at an MOI=1:250. The indicated combinations of DHA (25  $\mu$ M),  $\alpha$ T (10  $\mu$ M) and cholesterol (chol.; 13  $\mu$ M) was added to the cells at 6 hpi. The cells were photographed 72 hours after infection. As shown in Figure 9, foci were larger in cells treated with DHA +  $\alpha$ T and larger yet when treated with DHA +  $\alpha$ T + cholesterol, consistent with the view that the treatments accelerated virus spread from cell to cell.

**EXAMPLE 9**

[0075] Virus replication utilizes the energy and precursors for macromolecule synthesis provided by the host cell. These biosynthetic and energetic demands are particularly large during infection with herpes viruses. Previous work has shown that certain viruses institute their own metabolic program in infected cells that requires the use of carbon from glucose mainly in biosynthetic reactions instead of for energy production (reviewed in Yu et al., *Trends in Microbiology*, **19** (7):360-7, 2011. This process is coupled with glutaminolysis, a set of reactions that convert glutamine which is supplied to cells from the medium to  $\alpha$ -ketoglutarate, replenishing the TCA cycle and providing the energy required for viral replication.

[0076] Previously work has shown that the inhibition of sirtuins with siRNAs or drugs can enhance the yield of multiple viruses grown in cultured cells (Koyuncu, Shenk and Cristea, "Sirtuins as inhibitors of cytomegalovirus", PCT application filed 2/2012). Since  $\alpha$ -ketoglutarate is produced and metabolized in the mitochondrion and multiple sirtuins regulate processes in mitochondria, and, specifically, since sirtuin 4 is known to regulate the production of  $\alpha$ -ketoglutarate in mitochondria (Haigis et al., *Cell*, **126** (5):941-54, 2006), experiments were designed to determine whether the level of  $\alpha$ -ketoglutarate might become limiting in virus-infected cells and therefore limit the amount of virus produced. Thus, the experiments examined whether  $\alpha$ -ketoglutarate added to the medium of infected cells can influence the yield of a test virus.

[0077] It is known that  $\alpha$ -ketoglutarate is highly hydrophilic and cannot efficiently penetrate across plasma membrane of the cells. Therefore, a cell permeating derivative of  $\alpha$ -

ketoglutarate (dimethyl-  $\alpha$ -ketoglutarate,  $\alpha$ -kg, Willenborg et al., *Eur J Pharmacol*, **607** (1-3):41-6, 2009; Sigma) was used in all experiments.

[0078] The initial experiments were designed to test whether virus replication could be enhanced by supplementing the cells with  $\alpha$ -kg.

[0079] Briefly, MRC5 cells were grown in DMEM containing 10% fetal calf serum, 2 mM GlutaMAX(GIBCO® GlutaMAX™ media contains L-alanyl-L-glutamine, which substitutes for glutamine and prevents degradation and ammonia build-up even during long-term cultures)) at 35°C as described in the text. The cells were infected with a known amount of VZV-infected MRC5 cells at a ratio of 1 infected cell per 100 uninfected cells (MOI=1:100) in a glutamine free medium or a medium containing 2 mM glutamine (Glutamax) as indicated. In both cases, 10% fetal calf serum was included after infection. 6 hours after infection  $\alpha$ -kg or GlutaMAX was added to the cells at indicated concentrations. Either glutamine or GlutaMAX is acceptable for supplementation of growth media, and they can be used interchangeably for the purposes of our invention. 72 hours after infection, the cells were harvested into PSGC buffer, sonicated and the yield of cell free VZV quantified by standard plaque assay on ARPE-19 cells. The virus titers are the average of two independent infections. Star (\*) indicates that the virus titer at this concentration is below detection limit of the assay. NT – not treated.

[0080] More specifically, MRC5 fibroblasts (American Type Culture Collection; passage number 20 -25) were seeded in 100 mm dishes at a ratio of approximately 300,000 cells per dish. The cells were grown at 35°C in 15 ml medium (Dulbecco's Modified Eagle Medium, DMEM) containing 2 mM GlutaMAX, and 10 % fetal calf serum (FCS). Three days after seeding, the culture medium was replaced with 10 ml growth medium containing 50 mM sucrose as a stabilizer. The cells were further grown for 3 days and growth medium was replaced with fresh medium containing no sucrose and either 2 mM or no glutamine/GlutaMAX.

[0081] The cells were then infected with a known amount of VZV-infected MRC5 cells (MOI=1:100) and, following an incubation period of 6 hours to allow cells to settle, GlutaMAX or  $\alpha$ -kg was added at selected concentrations. Seventy two hours after infection, cells were washed twice with PBS, and incubated in 10 ml of PBS containing 50 mM ammonium chloride for 50 minutes at 4°C. The cells were harvested by scraping into 1 ml of PSGC buffer and sonicated in a bath-type sonicator for two rounds of 15 seconds with 15

second intervals. The cellular debris was removed by low-speed centrifugation, and the virus yield in the supernatant was quantified by plaque assay in ARPE-19 cells. The cell-free virus was frozen at -80°C for 1 day and kept in liquid nitrogen for long-term storage. For plaque assays, ARPE-19 cells (passage number 25 – 30) were seeded into 6-well dishes at ~300,000 cell/well.

[0082] Procedurally, the cells were incubated 2 days prior to infection at 37°C. During the time of infection, the cells were 70-80 % confluent, which is required for optimum infection. Two-hours after infection, the medium of the cells were replaced with methylcellulose overlay.

[0083] As is evident in Figure 10,  $\alpha$ -kg at 7 mM concentration increased the virus yield about 2.1 fold in the presence of 2 mM GlutaMAX. In the absence of GlutaMAX, inclusion of  $\alpha$ -kg at 7 mM enhanced the virus replication by a factor of ~2.8 fold when compared to the addition of 2 mM GlutaMAX. This indicates that GlutaMAX negatively affects the ability of  $\alpha$ -kg to enhance the replication of VZV. On the other hand,  $\alpha$ -kg at 2.5 and 1 mM were unable to support virus replication in the absence of GlutaMAX and VZV titers were substantially inhibited at these concentrations. Thus, a concentration of >2.5 mM  $\alpha$ -kg is required to optimally support the replication of VZV.

[0084] These results showed that a cell permeable derivative of  $\alpha$ -ketoglutarate, dimethyl- $\alpha$ -ketoglutarate ( $\alpha$ -kg), can be used to increase virus production in cultured cells. Examples of cell permeable  $\alpha$ -ketoglutarate derivatives include but are not limited to octyl- $\alpha$ -ketoglutarate and TFMB- $\alpha$ -ketoglutarate, in addition to the dimethyl derivative. These monoester derivatives of  $\alpha$ -ketoglutarate have been shown to efficiently enter the cells and to subsequently be cleaved by cytosolic esterases to yield  $\alpha$ -ketoglutarate (MacKenzie et al., *Mol. Cell. Biol.*, **27** (9): 3282-9, 2007).

## EXAMPLE 10

[0085] Experiments described above demonstrated a method for increasing the yield of virus production in cultured cells by supplementation of growth medium with certain fatty acids, scavenging compounds and cholesterol.

[0086] Among these, a combination of docosahexaenoic acid (DHA),  $\alpha$ -tocopherol ( $\alpha$ T), and cholesterol substantially increases VZV production.

[0087] MRC5 cells were infected with VZV-infected MRC5 cells at an MOI=1:50 in glutamine/GlutaMAX-free medium. GlutaMAX (NT; 2 mM), docosahexaenoic acid (DHA; 25  $\mu$ M),  $\alpha$ -tocopherol ( $\alpha$ -T; 10  $\mu$ M), cholesterol (chol.; 13  $\mu$ M), and  $\alpha$ -kg, (7 mM) were added on the cells at 6 hpi as indicated. 72 hours after infection, the cells were harvested into PSGC buffer, sonicated and the yield of cell free VZV quantified by standard plaque assay on ARPE-19 cells. The titers are the average of two independent infections.

[0088] In view of these results, experiments were designed to determine whether addition of this combination together with  $\alpha$ -kg further enhances virus replication.

[0089] As shown in Figure 11,  $\alpha$ -kg alone increased VZV yields by a factor of ~3.4 fold and inclusion of DHA plus  $\alpha$ -T plus cholesterol combination further enhanced the virus yields to approximately 4.8 fold. These results demonstrated that supplementing the cells with  $\alpha$ -ketoglutarate can be used along with fatty acids, scavenging compounds and cholesterol for the further enhancement of virus replication.

## EXAMPLE 11

[0090] In the next experiment, the possibility was tested whether supplementation with  $\alpha$ -kg might enhance the yield of VZV to a greater extent in glutamine/GlutaMAX-free medium supplemented with a 10,000 MW cutoff filter dialyzed fetal calf serum than in glutamine/GlutaMAX-free medium supplemented with normal, undialyzed fetal calf serum. The dialyzed serum would lack glutamine, which would be present at some level in normal, undialyzed serum.

[0091] MRC5 cells were grown in DMEM containing 10% fetal calf serum, 2 mM GlutaMAX at 35°C. The cells were infected VZV-infected MRC5 cells at a multiplicity of 1:250 in a glutamine/GlutaMAX-free medium containing either 10 % FCS (normal FCS) or 10 % dialyzed FCS. 6 hours after infection  $\alpha$ -kg or glutamax was added to the cells at indicated concentrations. 72 hours after infection, the cells were harvested into PSGC buffer, sonicated, and the yield of cell free VZV was quantified by standard plaque assay on ARPE-19 cells.

[0092] Results are shown in Figure 12. The yield of VZV was increased by a factor of ~3.7-fold in glutamine/GlutaMAX-free medium containing normal serum, and the yield of virus was increased by a factor of ~9.7 in glutamine-free medium supplemented with dialyzed serum. Thus, we conclude that removal of small, dialyzable molecules such as

glutamine from serum further enhances the production of VZV. Surprisingly, supplementation of GlutaMAX-containing medium with dialyzed serum also increased virus production – by a factor of ~3.2. This demonstrates another method by which the yield of virus can be increased, i.e., by dialysis of serum, which presumably removes an inhibitory constituent.

## EXAMPLE 12

[0093] The effect of  $\alpha$ -kg supplementation on the production of a human cytomegalovirus (HCMV) was then tested.

[0094] MRC5 fibroblasts were infected with the AD169 strain of HCMV at a multiplicity of 0.5 infectious units/cell in glutamine/GlutaMAX-free DMEM containing 10 % dialyzed FCS. The dialyzed serum was used to completely eliminate the glutamine in the culture medium. The cells received either 2 mM GlutaMAX, which served as a control, or 7 mM  $\alpha$ -kg. At 96 hours post infection, infectious virus in the medium was assayed by fluorescent focus assay using antibody to the HCMV IE1 protein.

[0095] Results are shown in Figure 13. Similar to VZV, the production of cell-free HCMV was increased by a factor of 4.3 fold by replacing glutamine with  $\alpha$ -kg. These results demonstrate that  $\alpha$ -ketoglutarate derivatives can be used for increasing the production of two different viruses, VZV and HCMV; and predict that a variety of viruses, including but not limited to herpesviruses, influenza viruses, poliovirus, rotavirus, hepatitis A virus, foot and mouth disease virus, rabies virus, parvovirus and adenovirus, would be similarly supported by supplementation with  $\alpha$ -ketoglutarate derivatives. In addition, other TCA cycle intermediates such as oxaloacetate, whose levels are influenced by the levels of  $\alpha$ -ketoglutarate could be used to facilitate the production of viruses in cultured cells either alone or in combination with  $\alpha$ -ketoglutarate.

[0096] These results indicate that supplementation of the medium with  $\alpha$ -ketoglutarate will enhance the production of additional viruses, including but not limited to herpes simplex virus, Epstein Barr virus, adenovirus, adeno-associated virus, hepatitis A virus, hepatitis C virus, Dengue virus, HIV, mumps virus, measles virus, rotavirus and parainfluenza virus.

[0097] Numerous modifications and variations in the invention as set forth in the above illustrative examples are expected to occur to those skilled in the art. Consequently only such limitations as appear in the appended claims should be placed on the invention.

**WHAT IS CLAIMED IS:**

1. A method for producing a virus comprising the step of culturing a host cell infected with a virus under conditions appropriate for producing the virus, wherein the conditions include  $\alpha$ -ketoglutarate, or a derivative thereof, in an amount and for a time effective to permit virus production.
2. The method of claim 1 wherein the virus is produced at in amount greater in the presence of  $\alpha$ -ketoglutarate, or the derivative thereof compared to virus produced in the method performed without  $\alpha$ -ketoglutarate, or the derivative thereof.
3. The method of claim 1 or 2 wherein the  $\alpha$ -ketoglutarate, or a derivative thereof is present at a concentration greater than 1.5 mM.
4. The method of any of the claims above wherein the  $\alpha$ -ketoglutarate, or the derivative thereof is present at a concentration greater than 1.6 mM, 1.7 mM, 1.8 mM, 1.9 mM, 2 mM, 2.1 mM, 2.2 mM, 2.3 mM, 2.4 mM, 2.5 mM, 2.6 mM, 2.7 mM, 2.8 mM, 2.9 mM, 3 mM, 3.1 mM, 3.2 mM, 3.3 mM, 3.4 mM, 3.5 mM, 3.6 mM, 3.7 mM, 3.8 mM, 3.9 mM, 4 mM, 4.1 mM, 4.2 mM, 4.3 mM, 4.4 mM, 4.5 mM, 4.6 mM, 4.7 mM, 4.8 mM, 4.9 mM, 5 mM, 5.1 mM, 5.2 mM, 5.3 mM, 5.4 mM, 5.5 mM, 5.6 mM, 5.7 mM, 5.8 mM, 5.9 mM, 6 mM, 6.1 mM, 6.2 mM, 6.3 mM, 6.4 mM, 6.5 mM, 6.6 mM, 6.7 mM, 6.8 mM, 6.9 mM, 7 mM, 7.1 mM, 7.2 mM, 7.3 mM, 7.4 mM, 7.5 mM, 7.6 mM, 7.7 mM, 7.8 mM, 7.9 mM, 8 mM, 8.1 mM, 8.2 mM, 8.3 mM, 8.4 mM, 8.5 mM, 8.6 mM, 8.7 mM, 8.8 mM, 8.9 mM, 9 mM, 9.1 mM, 9.2 mM, 9.3 mM, 9.4 mM, 9.5 mM, 9.6 mM, 9.7 mM, 9.8 mM, 9.9 mM, 10 mM or more.
5. The method of any of the claims above wherein the  $\alpha$ -ketoglutarate, or the derivative, is present at a concentration of less than 10 mM.
6. The method of any of the claims above wherein the  $\alpha$ -ketoglutarate, or the derivative, is present at a concentration of less than 2.3 mM, 2.4 mM, 2.5 mM, 2.6 mM, 2.7 mM, 2.8 mM, 2.9 mM, 3 mM, 3.1 mM, 3.2 mM, 3.3 mM, 3.4 mM, 3.5 mM, 3.6 mM, 3.7 mM, 3.8 mM, 3.9 mM, 4 mM, 4.1 mM, 4.2 mM, 4.3 mM, 4.4 mM, 4.5 mM, 4.6 mM, 4.7 mM, 4.8 mM, 4.9 mM, 5 mM, 5.1 mM, 5.2 mM, 5.3 mM, 5.4 mM, 5.5 mM, 5.6 mM, 5.7 mM, 5.8 mM, 5.9 mM, 6 mM, 6.1 mM, 6.2 mM, 6.3 mM, 6.4 mM, 6.5 mM, 6.6 mM, 6.7 mM, 6.8 mM, 6.9 mM, 7 mM, 7.1 mM, 7.2 mM, 7.3 mM, 7.4 mM, 7.5 mM, 7.6 mM, 7.7

mM, 7.8 mM, 7.9 mM, 8 mM, 8.1 mM, 8.2 mM, 8.3 mM, 8.4 mM, 8.5 mM, 8.6 mM, 8.7 mM, 8.8 mM, 8.9 mM, 9 mM, 9.1 mM, 9.2 mM, 9.3 mM, 9.4 mM, 9.5 mM, 9.6 mM, 9.7 mM, 9.8 mM, 9.9 mM or 10 mM.

7. The method of any of the claims above wherein the  $\alpha$ -ketoglutarate derivative is selected from the group consisting of.

8. The method of any of the claims above wherein  $\alpha$ -ketoglutarate is present along with an  $\alpha$ -ketoglutarate derivative.

9. The method of any one of the claims above wherein more than one derivative of  $\alpha$ -ketoglutarate is present.

10. The method any of the claims above, wherein the conditions further include a fatty acid in an amount and for a time effective to permit virus production.

11. The method of any of the claims above wherein the virus is produced at in amount greater in the presence of the fatty acid compared to virus produced in the method performed without the fatty acid.

12. The method of any of the claims above wherein the conditions include the presence of a fatty acid and cholesterol.

13. The method of claim 12 wherein the virus is produced in an amount greater in the presence of the fatty acid and cholesterol compared to virus produced in the method performed without the fatty acid and cholesterol.

14. The method of any of the claims above wherein the conditions further include a scavenging compound.

15. The method of claim 14 wherein the virus is produced in an amount greater in the presence of the scavenger compound compared to virus produced in the method performed without the scavenger compound.

16. The method of any one of claims 10-15 wherein the conditions include no more than one fatty acid.

17. The method of any one of claims 10-15 wherein the conditions include no more than two fatty acids.
18. The method of any one of claims 10-15 wherein the conditions include no more than three fatty acids.
19. The method of any one of claims 10-15 wherein the conditions include no more than four fatty acids.
20. The method of any one of claims 10-15 wherein the conditions include at least two different fatty acids.
21. The method of any one of claims 10-15 wherein the conditions include at least three different fatty acids.
22. The method of any one of claims 10-15 wherein the conditions include at least four different fatty acids.
23. The method of any one of claims 10-15 wherein the conditions include four or more different fatty acids.
24. The method of any one of claims 10-23 wherein the fatty acid or each fatty acid is essentially homogenous when introduced into culture.
25. The method of any of claims 1-24 further comprising the step of isolating said virus from medium of cell growth.
26. The method of any of claims 1-25 further comprising the step of isolating the virus from the host cell.
27. The method of any one of claims 1-26 further comprising the step of infecting the host cells with the virus.
28. The method of any of claims 1-27 further comprising the step of infecting the host cells by co-cultivating the host cells with the virus infected cells.
29. The method of any one of claims 1-28 further comprising the step of growing the host cells to about 80% confluence, about 70% confluence, about 60% confluence, about 50% confluence, or less than 50% confluence prior to infecting the host cells with the virus.

30. The method of any one of claims 1-28 further comprising the step of growing the host cells to confluence or 90% confluence prior to infecting the host cells with the virus.

31. The method of any one of claims 1-30 further comprising the steps of culturing the host cells after infecting the host cells with the virus.

32. The method of any one of claims 1-31 further comprising the step of adding or changing medium of growth for the host cells prior to isolating the virus.

33. The method of any one of claims 27-32 further comprising the step of incubating the host cells with an infecting virus for an adsorption period.

34. The method of claim 32 further comprising the step of introducing the fatty acid, cholesterol and/or scavenging compound during the step of adding or changing the medium.

35. The method of any one of claims 27-32 further comprising the step of introducing the fatty acid, cholesterol and/or scavenging compound prior to infecting the host cell with the virus or with virus infected cells.

36. The method of any one of claims 27-32 further comprising the step of introducing the fatty acid, cholesterol and/or scavenging compound after infecting the host cell with the virus.

37. The method of any one of claims 1-36 further comprising the step of introducing the fatty acid, cholesterol and/or scavenging compound at more than one time during the step of culturing the cells.

38. The method of any one of claims 1-37 further comprising the step freezing the host cells prior to isolating the virus.

39. The method of any one of claims 1-37 further comprising the step isolating the virus without freezing the host cells.

40. The method of claim 39 further comprising the step disrupting the host cells to isolate the virus.

41. The method of claim 40 wherein disrupting the host cells is carried out using a French press, sonication, or freeze/thaw cycling.

42. The method of any one of claims 1-41 wherein the host cell is infection-susceptible to the virus.

43. The method of any one of claims 1-42 wherein the host cell is mammalian.

44. The method of any one of claims 1-43 wherein the host cell is human.

45. The method of any one of claims 1-44 wherein the host cell is a fibroblast cell or an epithelial cell.

46. The method of any one of claims 1-45 wherein the host cell is an MRC5 cell, a retinal cell or an ARPE-19 cell.

47. The method of any one of claims 1-46 wherein the virus is an enveloped virus

48. The method of any one of claims 1-46 wherein the virus is an enveloped DNA virus or an enveloped RNA virus.

49. The method of any one of claims 1-46 wherein the virus is a herpes virus.

50. The method of any one of claims 1-46 wherein the virus is an alpha family herpes virus.

51. The method of any one of claims 1-46 wherein the virus is a beta family herpes virus.

52. The method of any one of claims 1-46 wherein the virus is an gamma family herpes virus.

53. The method of any one of claims 1-46 wherein the virus is VZV.

54. The method of any one of claims 1-46 wherein the virus is CMV.

55. The method of any one of claims 1-46 wherein the virus is an RNA virus, a nonenveloped RNA virus, an enveloped RNA virus, a DNA virus, a nonenveloped DNA virus, and enveloped DNA virus, a pox virus, a picorna virus, poliovirus, rhinovirus, hepatitis

A virus, foot and mouth disease virus, influenza virus, herpes simplex virus, Epstein Barr virus, hepatitis C virus, Dengue virus, HIV, mumps virus, measles virus, rotavirus and/or parainfluenza virus.

56. The method of any one of claims 1-55 wherein cholesterol is a cholesterol derivative.

57. The method of any one of claims 1-56 wherein cholesterol is a cholesterol ester.

58. The method of any one of claims 1-57 wherein the fatty acid is a long chain fatty acid or a very long chain fatty acid.

59. The method of any one of claims 1-58 wherein the fatty acid is an omega-3 fatty acid.

60. The method of any one of claims 1-59 wherein the fatty acid is an omega-6 fatty acid.

61. The method of any one of claims 1-60 wherein the fatty acid is a naturally-occurring fatty acid.

62. The method of any one of claims 1-60 wherein the fatty acid is a derivative of a naturally-occurring fatty acid.

63. The method of claim 62 wherein the fatty acid is a non-naturally-occurring fatty acid.

64. The method of any one of claims 1-63 wherein the fatty acid is a free fatty acid.

65. The method of any one of claims 1-64 wherein the fatty acid is a fatty acid ester.

66. The method of any one of claims 1-65 wherein the fatty acid is a fatty acid derivative.

67. The method of claim 66 wherein the fatty acid derivative is a triglyceride.

68. The method of claim 66 wherein the fatty acid derivative is a diglyceride.
69. The method of claim 66 wherein the fatty acid derivative is a monoglyceride.
70. The method of claim 66 wherein the fatty acid derivative is a phospholipid.
71. The method of any one of claims 1-70 wherein the fatty acid has at least 18 carbon.
72. The method of any one of claims 1-71 wherein the fatty acid has at least 20 carbons.
73. The method of any one of claims 1-72 wherein the fatty acid has at least 22 carbons.
74. The method of any one of claims 1-73 wherein the fatty acid has at least 24 carbons.
75. The method of any one of claims 1-74 wherein the fatty acid has at least 26 carbons.
76. The method of any one of claims 1-75 wherein the fatty acid has at least 28 carbons.
77. The method of any one of claims 1-76 wherein the fatty acid has at least 30 carbons.
78. The method of any one of claims 1-77 wherein the fatty acid has at least 32 carbons.
79. The method of any one of claims 1-78 wherein the fatty acid has at least 34 carbons.
80. The method of any one of claims 1-79 wherein the fatty acid has at least 36 carbons.
81. The method of any one of claims 1-80 wherein the fatty acid has at least 38 carbons.

82. The method of any one of claims 1-81 wherein the fatty acid has at least 40 carbons.
83. The method of any one of claims 1-82 wherein the fatty acid is saturated.
84. The method of any one of claims 1-82 wherein the fatty acid is unsaturated.
85. The method of claim 84 wherein the fatty acid is polyunsaturated.
86. The method of any one of claims 84-85 wherein the fatty acid has 1 or more double bonds.
87. The method of any one of claims 84-86 wherein the fatty acid has 2 or more double bonds.
88. The method of any one of claims 84-87 wherein the fatty acid has 3 or more double bonds.
89. The method of any one of claims 84-88 wherein the fatty acid has 4 or more double bonds.
90. The method of any one of claims 84-89 wherein the fatty acid has 5 or more double bonds.
91. The method of any one of claims 84-90 wherein the fatty acid has 6 or more double bonds.
92. The method of any one of claims 84-91 wherein the fatty acid has 7 or more double bonds.
93. The method of any one of claims 84-92 wherein the fatty acid has 8 or more double bonds.
94. The method of any one of claims 84-93 wherein the fatty acid has 9 or more double bonds.
95. The method of any one of claims 84-94 wherein the fatty acid has 10 or more double bonds.

96. The method of any one of claims 84-95 wherein the fatty acid has 11 or more double bonds.
97. The method of any one of claims 84-96 wherein the fatty acid has 12 or more double bonds.
98. The method of any one of claims 10-58 wherein the fatty acid is selected from the group consisting of: linoleic acid (LA),  $\alpha$ -linolenic acid (LLA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), arachidonic acid (AA), hexacosanoic acid (HSA) and octacosanoic acid, OSA)
99. The method of any one of claims 1-98 wherein the fatty acid and/or cholesterol is formulated in a mixture that improves delivery to and/or uptake in cells.
100. The method of claim 99 wherein fatty acid and/or cholesterol is associated with a polymer.
101. The method of claim 100 wherein the polymer is a protein or a synthetic polymer.
102. The method of claim 99 wherein fatty acid and/or cholesterol is associated with a small molecule.
103. The method of any one of claims 14-102 wherein the scavenging compound is a carbonyl scavenging compound or a free radical scavenging compound.
104. The method of any one of claims 14-103 further comprising a carbonyl scavenging compound and a free radical scavenging compound.
105. The method of any one of claims 14-104 wherein the scavenging compound is selected from the group consisting of aminoguanidine, alpha-tocopherol, hydralazine, glycosylisovitexin, N-acetyl-cystein, metformin, penicillamine, pyridoxamine, edaravone (EDA), tenilsetam, lipoic acid, 3,3-dimethyl-D-cysteine (DMC), L- 3,3-dimethyl-D-cysteine (L-DMC), N-acetyl-3,3-dimethyl-D-cysteine (ADMC), N<sup>α</sup>-acetyl-L-cysteine (NAC), 3,3-dimethyl-D-cysteine-disulfide (DMCSS), S-methyl-DMC (SMDMC), L-cysteine (CYS), L-cysteine-O-methylester (CYSM), 3,3-dimethyl-D-cysteine-methylester (DMCM), 3-methyl-3-ethyl-D-cysteine (MEC), semicarbazide hydrochloride SC (hydrazine carboxamide), 1,1-

dimethyl-biguanide hydrochloride (DMBG), N-tertbutylhydroxylamine(BHA), a flavonoid, a flavanol, epicatechin, a flavanone, naringenin, a flavonol, quercetin, a flavones, luteolin, an isoflavone, genistein, an anthocyanidin, cyanidin, a phenol/ phenolic acid, a flavan-3-ol compound, procyanidins B1 (9.8), procyanidins B2, (+)-catechin, (–)-epicatechin, caftaric acid, caffeic acid, and kaempferol.

106. The method of any one of claims 10-105 wherein the fatty acid is present at a concentration of at least 5  $\mu\text{M}$ , at least 10  $\mu\text{M}$ , at least 15  $\mu\text{M}$ , at least 20  $\mu\text{M}$ , at least 25 $\mu\text{M}$ , at least 30  $\mu\text{M}$ , at least 35  $\mu\text{M}$ , at least 40  $\mu\text{M}$ , at least 45  $\mu\text{M}$ , at least 50  $\mu\text{M}$ , at least 55  $\mu\text{M}$ , at least 60  $\mu\text{M}$ , at least 65  $\mu\text{M}$ , at least 70  $\mu\text{M}$ , at least 75  $\mu\text{M}$ , at least 80  $\mu\text{M}$ , at least 85 $\mu\text{M}$ , at least 90  $\mu\text{M}$ , at least 95  $\mu\text{M}$ , at least 100  $\mu\text{M}$ , at least 110  $\mu\text{M}$ , at least 120  $\mu\text{M}$ , at least 130  $\mu\text{M}$ , at least 140  $\mu\text{M}$ , at least 150  $\mu\text{M}$  or more, and wherein the fatty acid is present at a concentration of 500  $\mu\text{M}$  or less, or at a concentration that is not toxic to the host cell.

107. The method of any one of claims 12-106 wherein cholesterol is present at a concentration of at least 5  $\mu\text{M}$ , at least 10  $\mu\text{M}$ , at least 15  $\mu\text{M}$ , at least 20  $\mu\text{M}$ , at least 25 $\mu\text{M}$ , at least 30  $\mu\text{M}$ , at least 35  $\mu\text{M}$ , at least 40  $\mu\text{M}$ , at least 45  $\mu\text{M}$ , at least 50  $\mu\text{M}$ , at least 55  $\mu\text{M}$ , at least 60  $\mu\text{M}$ , at least 65  $\mu\text{M}$ , at least 70  $\mu\text{M}$ , at least 75  $\mu\text{M}$ , at least 80  $\mu\text{M}$ , at least 85 $\mu\text{M}$ , at least 90  $\mu\text{M}$ , at least 95  $\mu\text{M}$ , at least 100  $\mu\text{M}$ , at least 110  $\mu\text{M}$ , at least 120  $\mu\text{M}$ , at least 130  $\mu\text{M}$ , at least 140  $\mu\text{M}$ , at least 150  $\mu\text{M}$  or more and wherein cholesterol is present at a concentration of 500  $\mu\text{M}$  or less, or at a concentration that is not toxic to the host cell.

108. The method of any one of claims 14-107 wherein the scavenging compound is present at a concentration of at least 1  $\mu\text{M}$ , at least 2  $\mu\text{M}$ , at least 3  $\mu\text{M}$ , at least 4  $\mu\text{M}$ , at least 5  $\mu\text{M}$ , at least 6  $\mu\text{M}$ , at least 7  $\mu\text{M}$ , at least 8  $\mu\text{M}$ , at least 9  $\mu\text{M}$ , at least 10  $\mu\text{M}$ , at least 15  $\mu\text{M}$ , at least 20  $\mu\text{M}$ , at least 25 $\mu\text{M}$ , at least 30  $\mu\text{M}$ , at least 35  $\mu\text{M}$ , at least 40  $\mu\text{M}$ , at least 45  $\mu\text{M}$ , at least 50  $\mu\text{M}$ , at least 55  $\mu\text{M}$ , at least 60  $\mu\text{M}$ , at least 65  $\mu\text{M}$ , at least 70  $\mu\text{M}$ , at least 75  $\mu\text{M}$ , at least 80  $\mu\text{M}$ , at least 85 $\mu\text{M}$ , at least 90  $\mu\text{M}$ , at least 95  $\mu\text{M}$ , at least 100  $\mu\text{M}$ , at least 110  $\mu\text{M}$ , at least 120  $\mu\text{M}$ , at least 130  $\mu\text{M}$ , at least 140  $\mu\text{M}$ , at least 150  $\mu\text{M}$  or more, and wherein the scavenging compound is present at a concentration of 500  $\mu\text{M}$  or less, or at a concentration that is not toxic to the host cell.

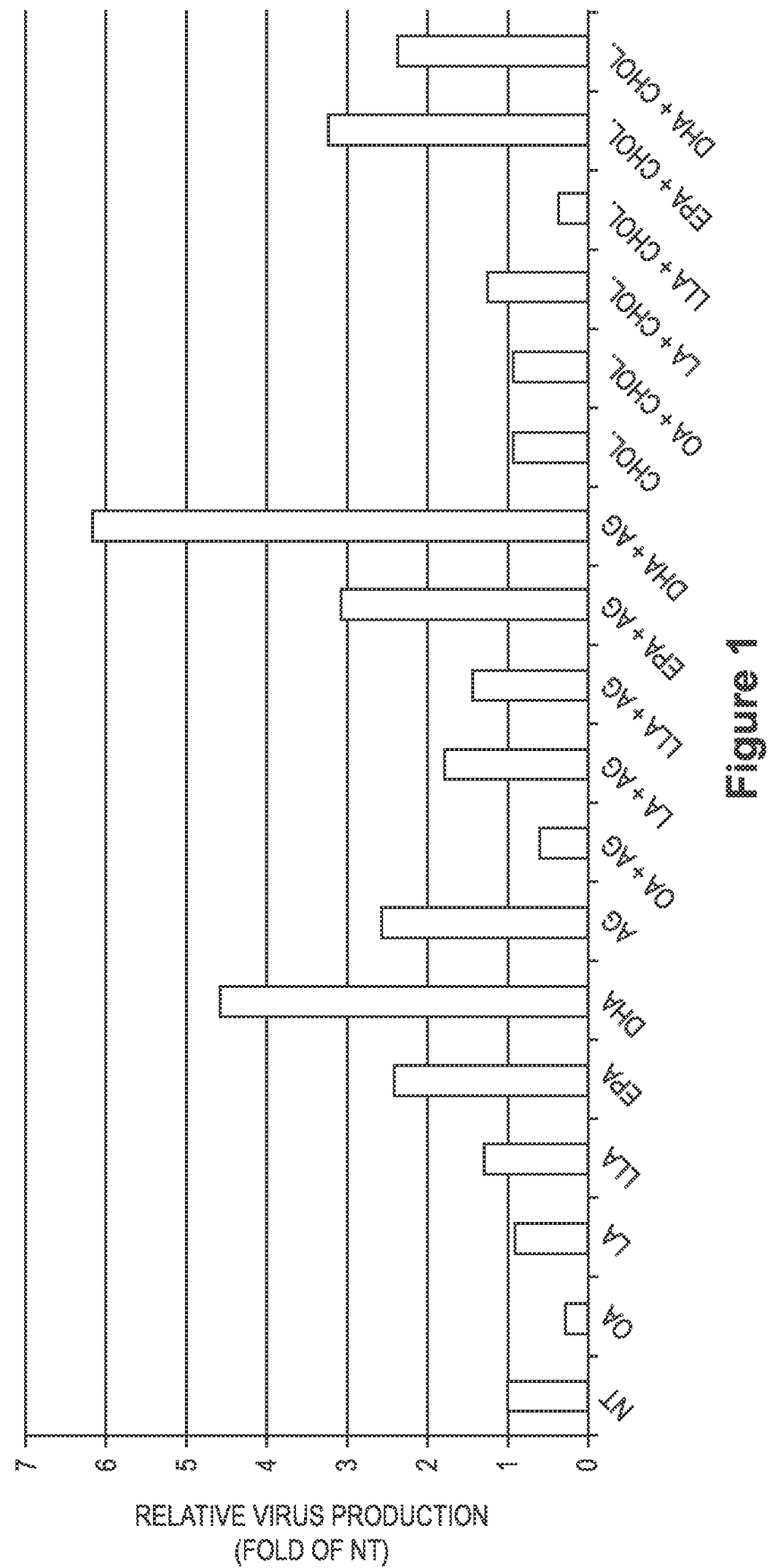
109. The method of any one of claims 10-105, 107 and 108 wherein the fatty acid is present at a concentration of no more than 5  $\mu\text{M}$ , no more than 10  $\mu\text{M}$ , no more than 15  $\mu\text{M}$ , no more than 20  $\mu\text{M}$ , no more than 25 $\mu\text{M}$ , no more than 30  $\mu\text{M}$ , no more than 35  $\mu\text{M}$ , no

more than 40  $\mu\text{M}$ , no more than 45  $\mu\text{M}$ , no more than 50  $\mu\text{M}$ , no more than 55  $\mu\text{M}$ , no more than 60  $\mu\text{M}$ , no more than 65  $\mu\text{M}$ , no more than 70  $\mu\text{M}$ , no more than 75  $\mu\text{M}$ , no more than 80  $\mu\text{M}$ , no more than 85  $\mu\text{M}$ , no more than 90  $\mu\text{M}$ , no more than 95  $\mu\text{M}$ , no more than 100  $\mu\text{M}$ , no more than 110  $\mu\text{M}$ , no more than 120  $\mu\text{M}$ , no more than 130  $\mu\text{M}$ , no more than 140  $\mu\text{M}$ , or no more than 150  $\mu\text{M}$ .

110. The method of any one of claims 12-106, 108 and 109 wherein cholesterol is present at a concentration of no more than 5  $\mu\text{M}$ , no more than 10  $\mu\text{M}$ , no more than 15  $\mu\text{M}$ , no more than 20  $\mu\text{M}$ , no more than 25  $\mu\text{M}$ , no more than 30  $\mu\text{M}$ , no more than 35  $\mu\text{M}$ , no more than 40  $\mu\text{M}$ , no more than 45  $\mu\text{M}$ , no more than 50  $\mu\text{M}$ , no more than 55  $\mu\text{M}$ , no more than 60  $\mu\text{M}$ , no more than 65  $\mu\text{M}$ , no more than 70  $\mu\text{M}$ , no more than 75  $\mu\text{M}$ , no more than 80  $\mu\text{M}$ , no more than 85  $\mu\text{M}$ , no more than 90  $\mu\text{M}$ , no more than 95  $\mu\text{M}$ , no more than 100  $\mu\text{M}$ , no more than 110  $\mu\text{M}$ , no more than 120  $\mu\text{M}$ , no more than 130  $\mu\text{M}$ , no more than 140  $\mu\text{M}$ , or no more than 150  $\mu\text{M}$ .

111. The method of any one of claims 14-107, 109, and 110 wherein the scavenging compound is present at a concentration of no more than 5  $\mu\text{M}$ , no more than 10  $\mu\text{M}$ , no more than 15  $\mu\text{M}$ , no more than 20  $\mu\text{M}$ , no more than 25  $\mu\text{M}$ , no more than 30  $\mu\text{M}$ , no more than 35  $\mu\text{M}$ , no more than 40  $\mu\text{M}$ , no more than 45  $\mu\text{M}$ , no more than 50  $\mu\text{M}$ , no more than 55  $\mu\text{M}$ , no more than 60  $\mu\text{M}$ , no more than 65  $\mu\text{M}$ , no more than 70  $\mu\text{M}$ , no more than 75  $\mu\text{M}$ , no more than 80  $\mu\text{M}$ , no more than 85  $\mu\text{M}$ , no more than 90  $\mu\text{M}$ , no more than 95  $\mu\text{M}$ , no more than 100  $\mu\text{M}$ , no more than 110  $\mu\text{M}$ , no more than 120  $\mu\text{M}$ , no more than 130  $\mu\text{M}$ , no more than 140  $\mu\text{M}$ , or no more than 150  $\mu\text{M}$ .

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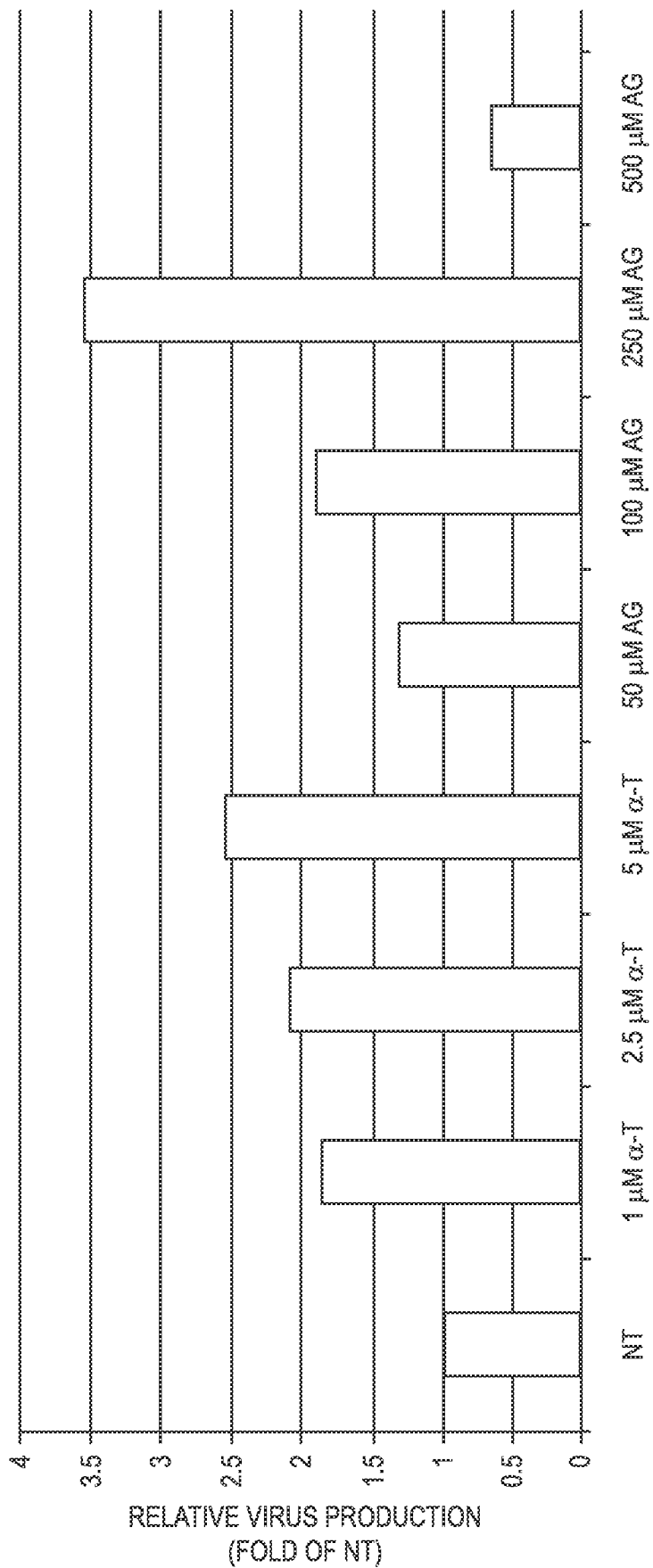


Figure 2

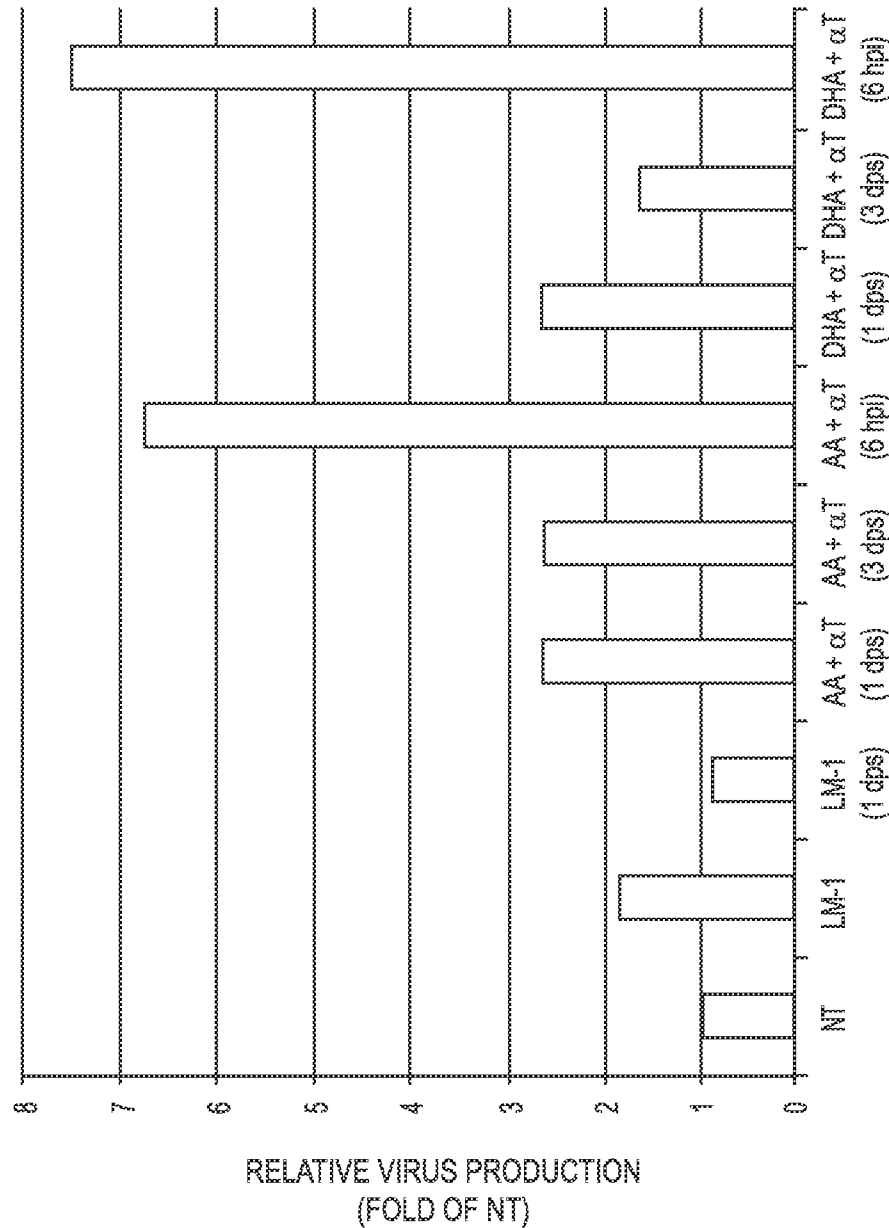
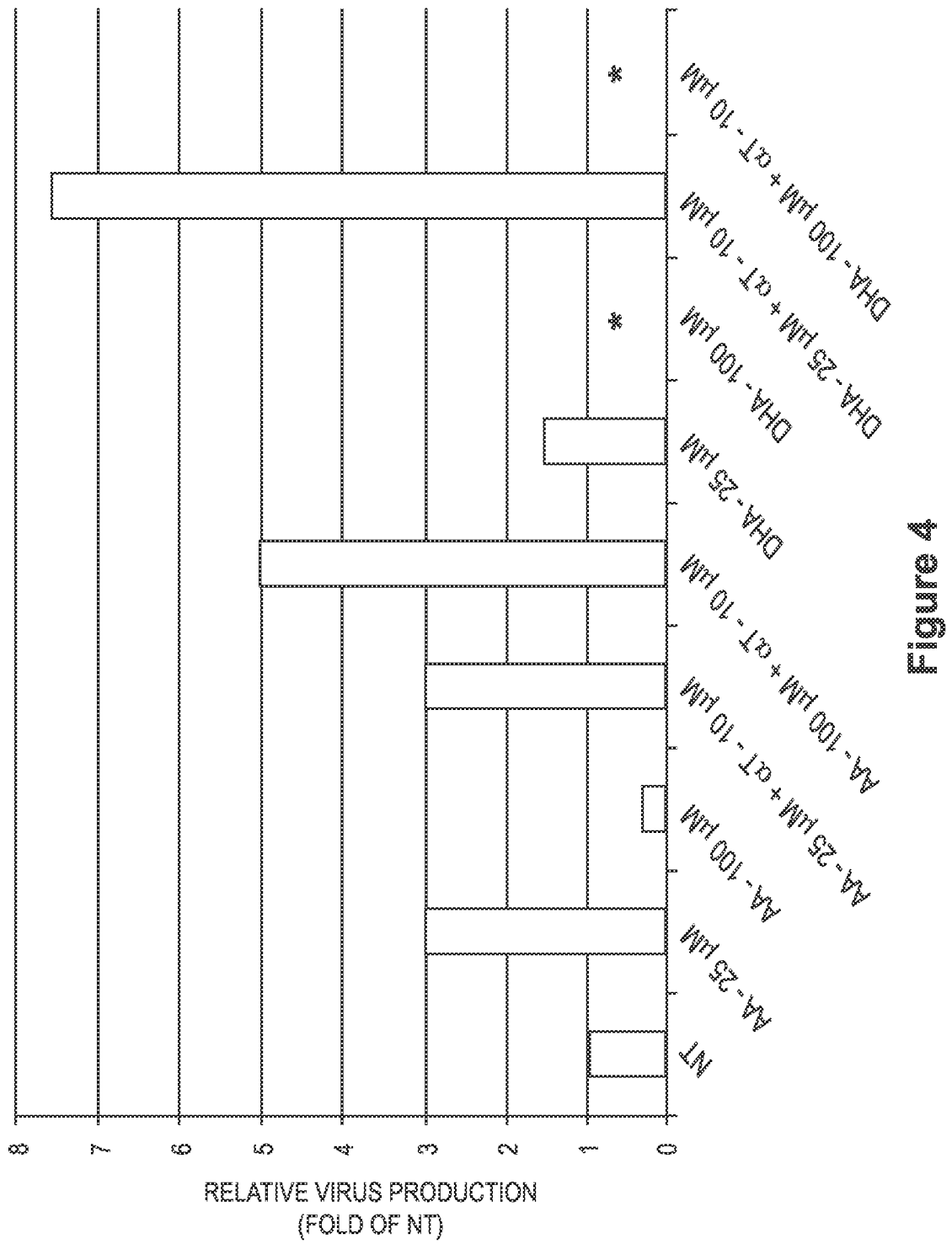


Figure 3

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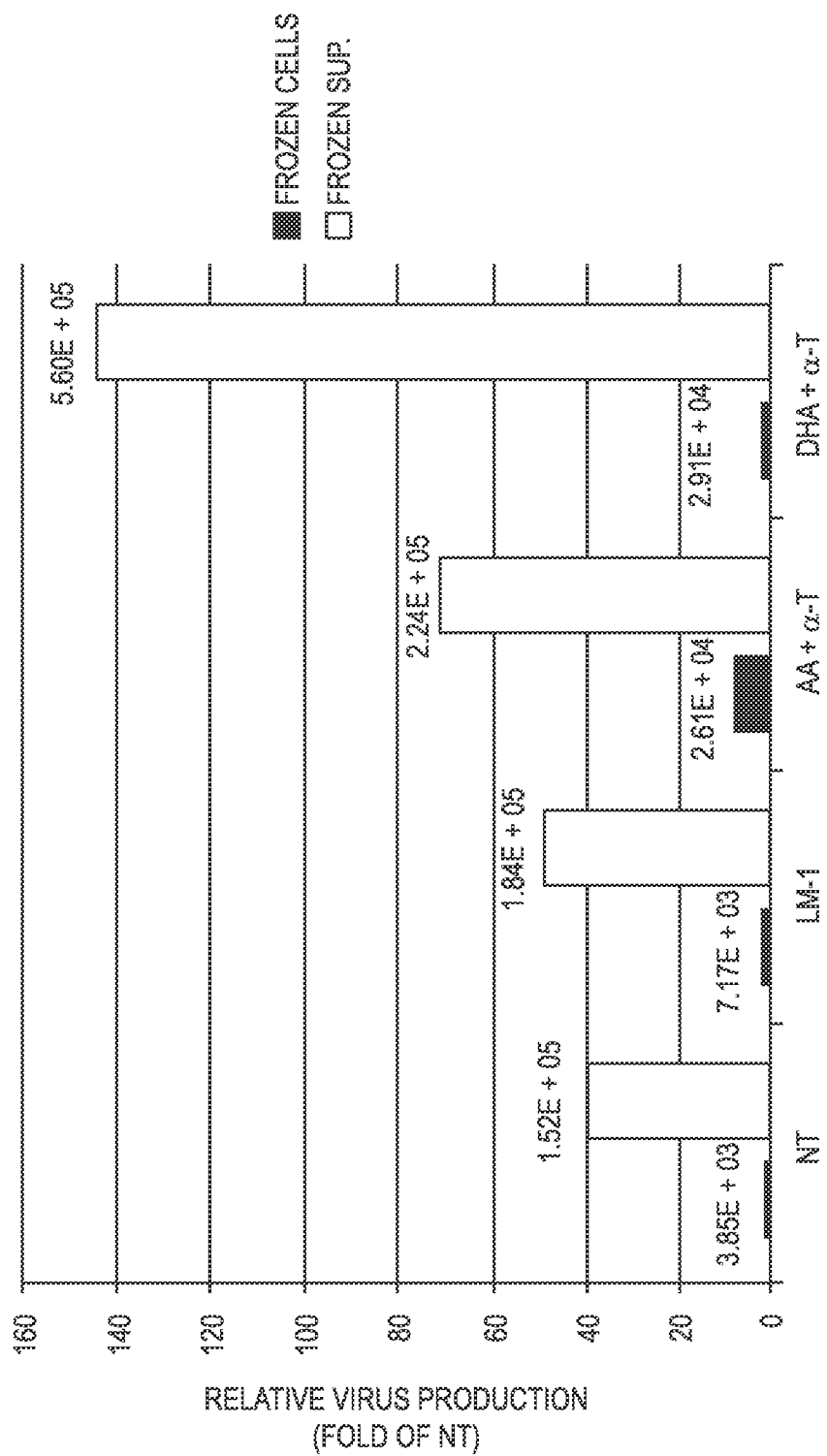
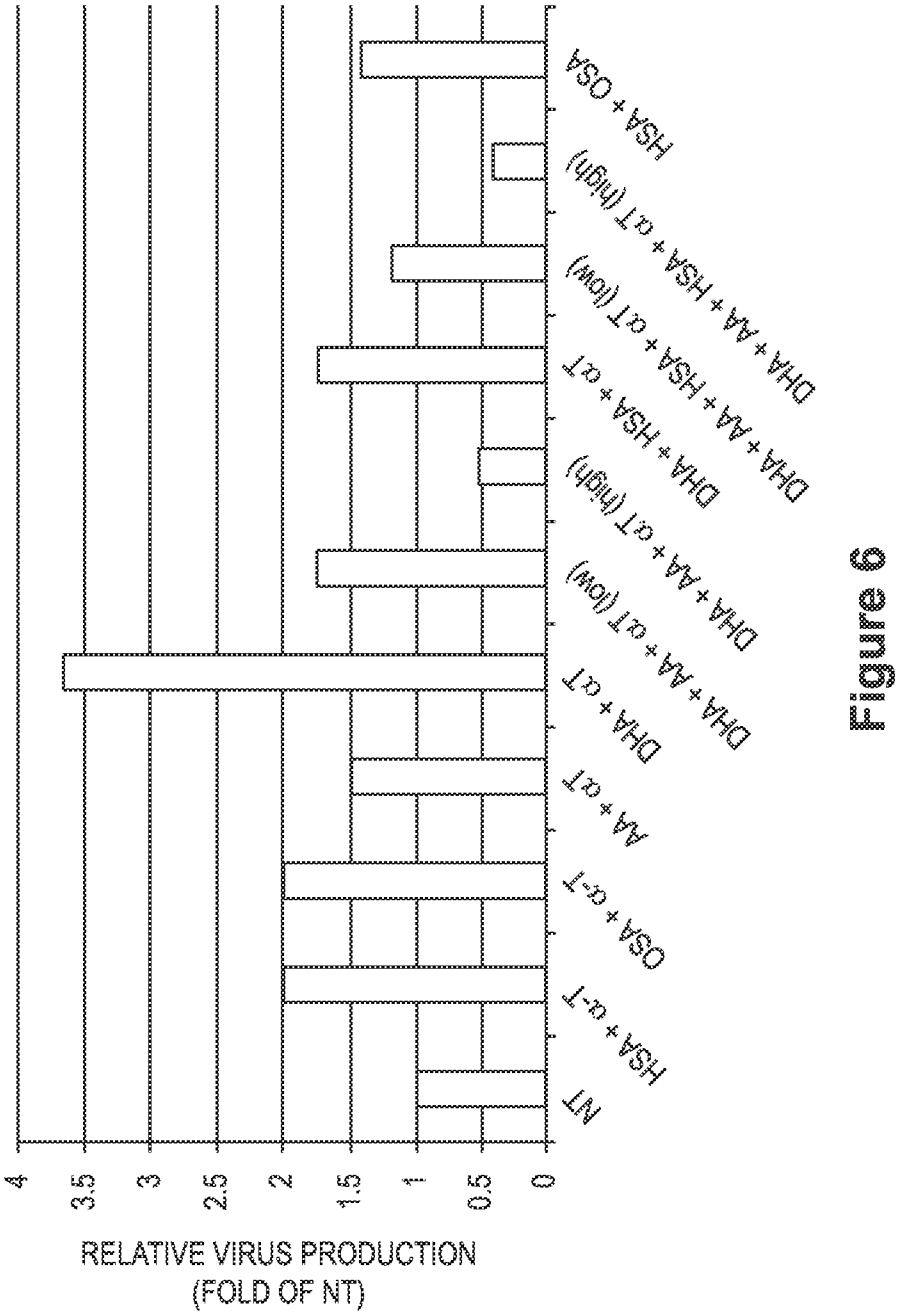


Figure 5



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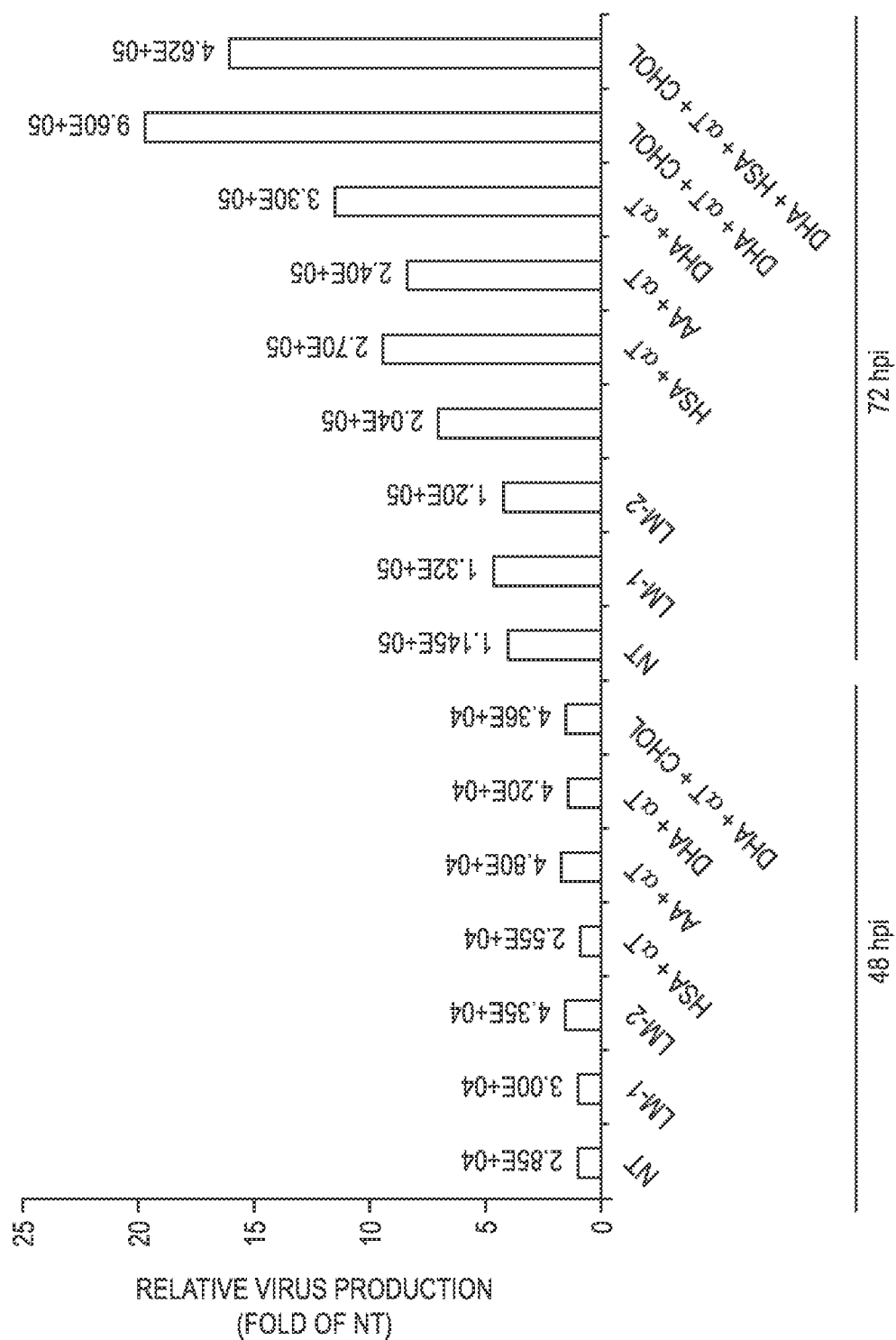


Figure 7

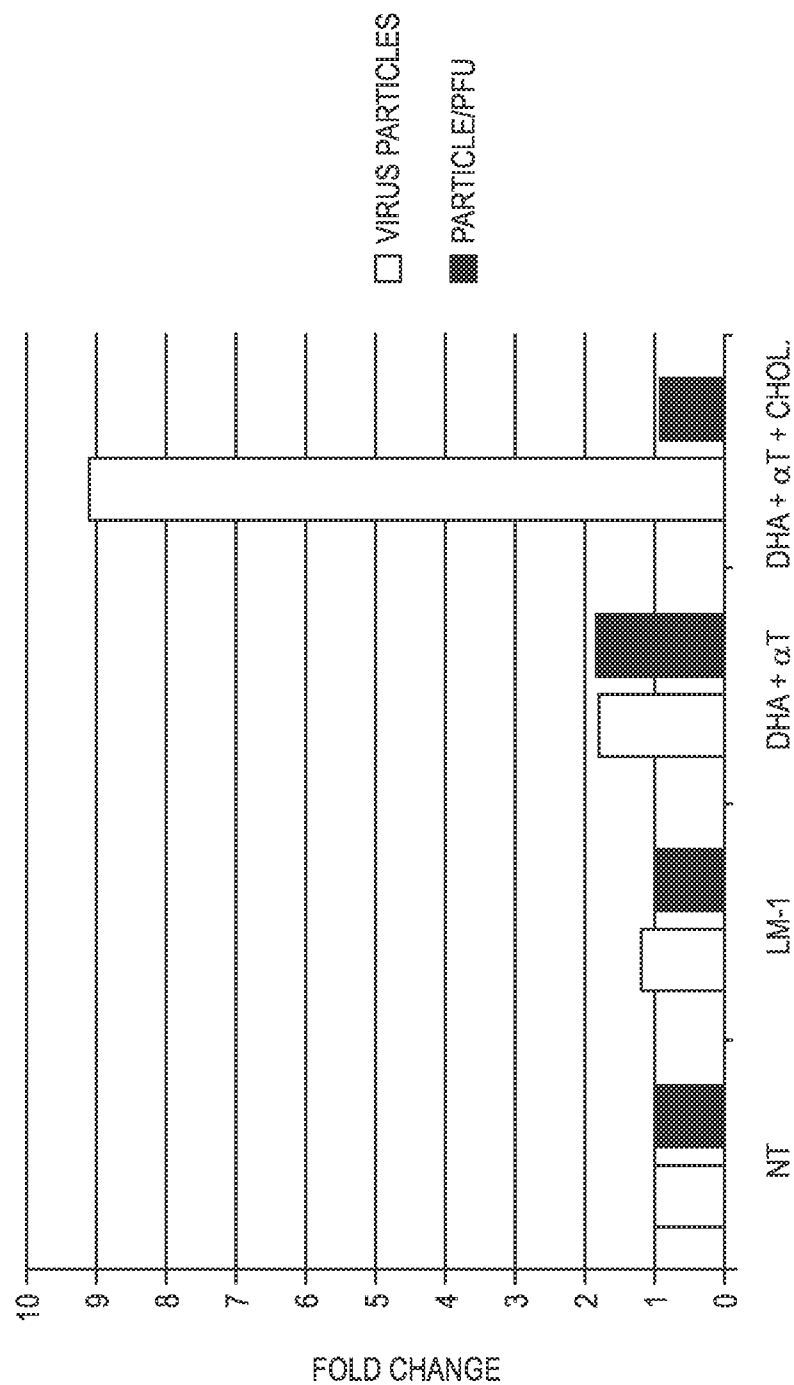


Figure 8

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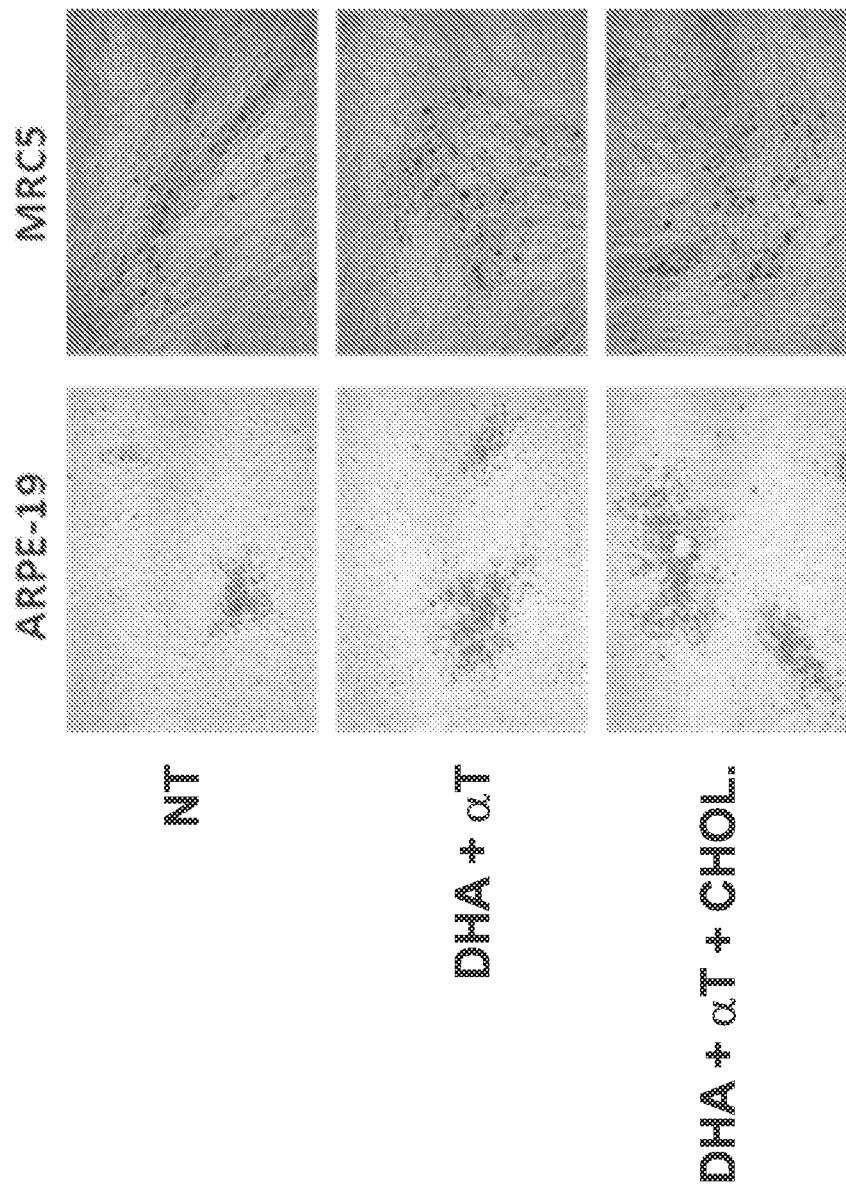


Figure 9

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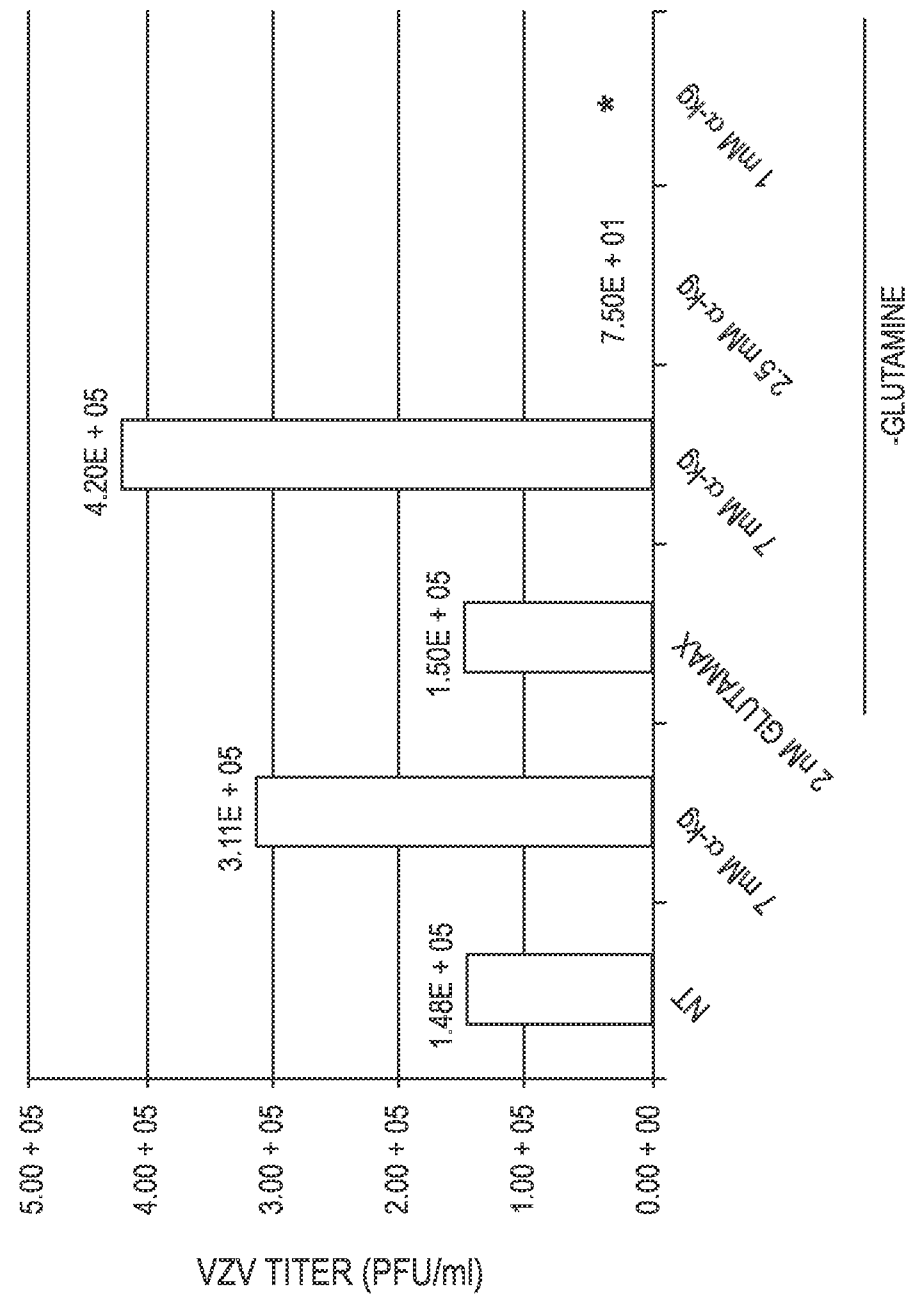


Figure 10

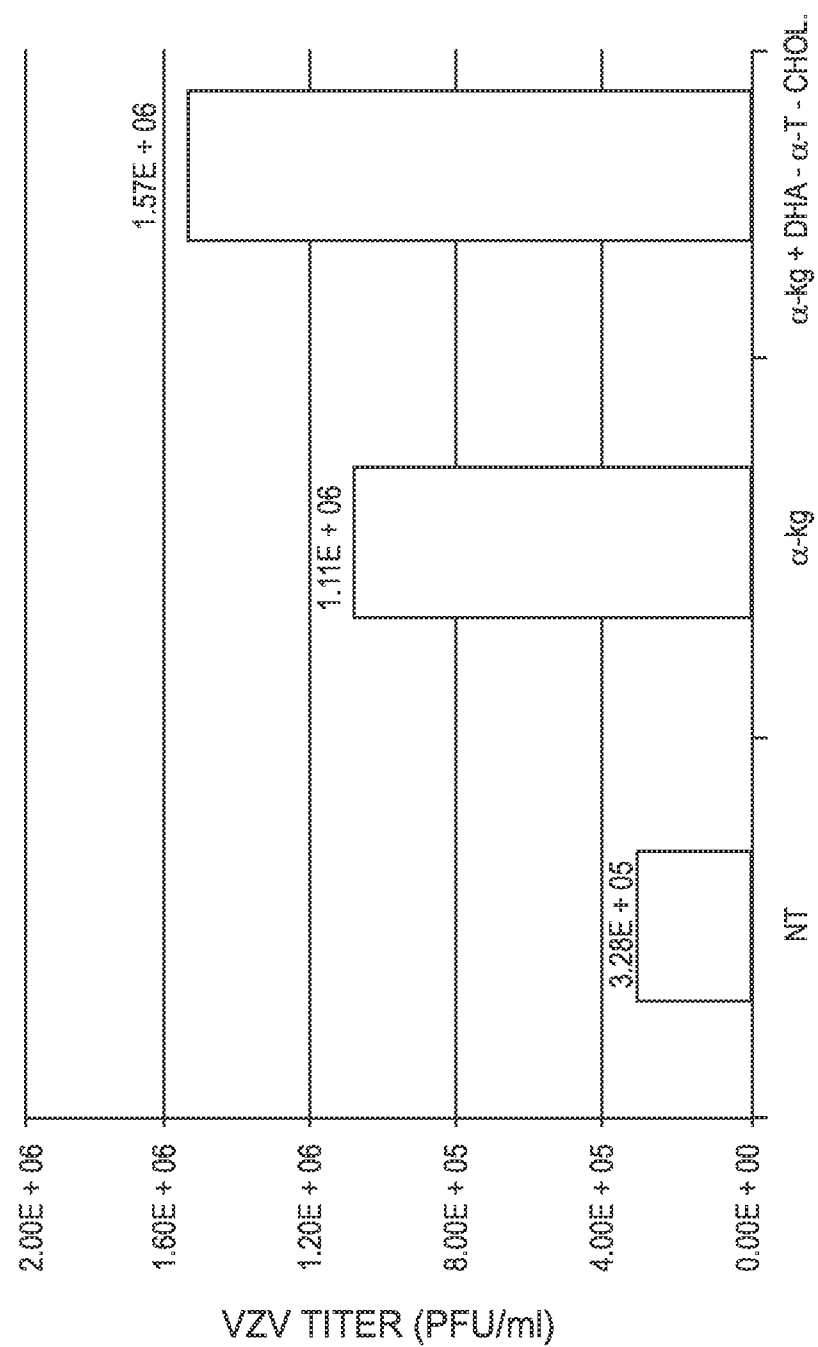


Figure 11

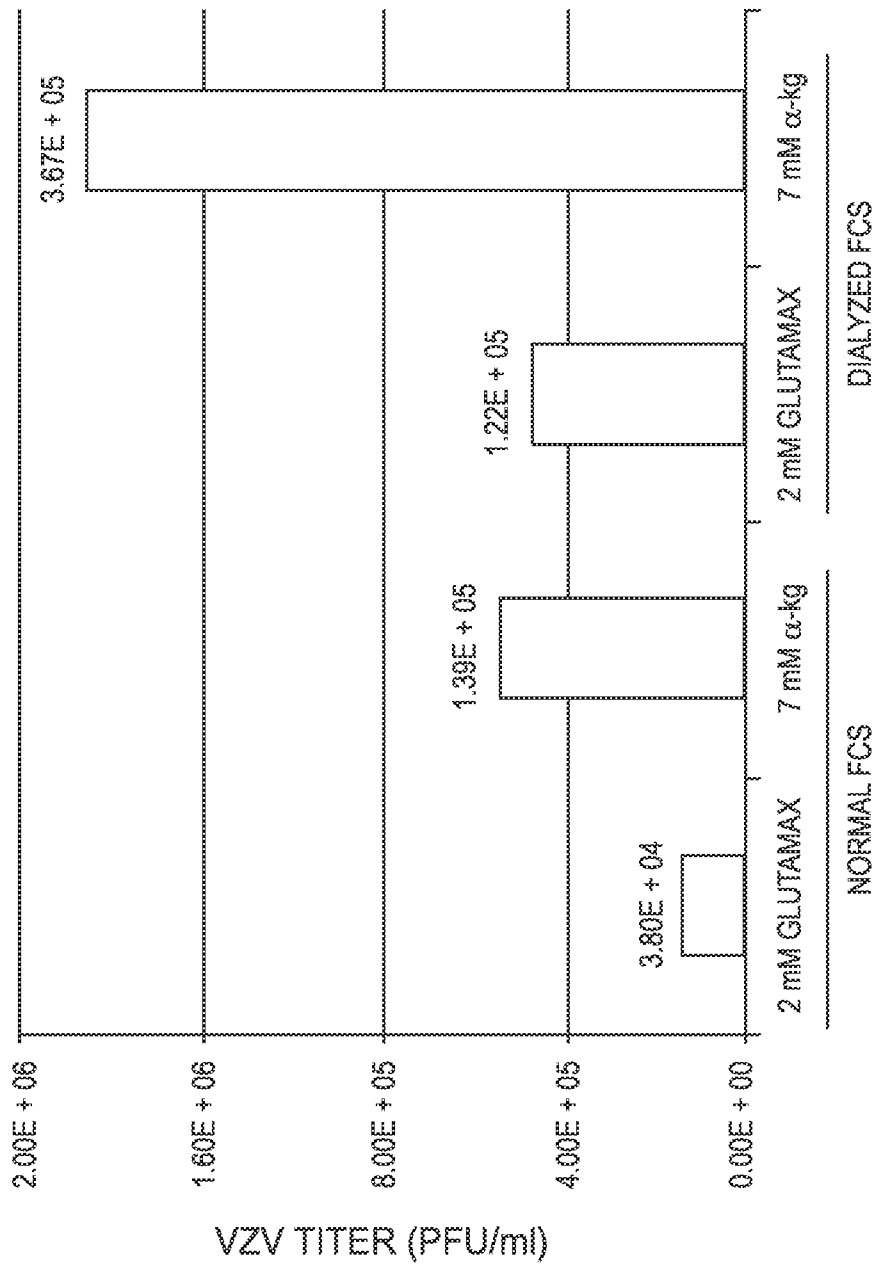


Figure 12

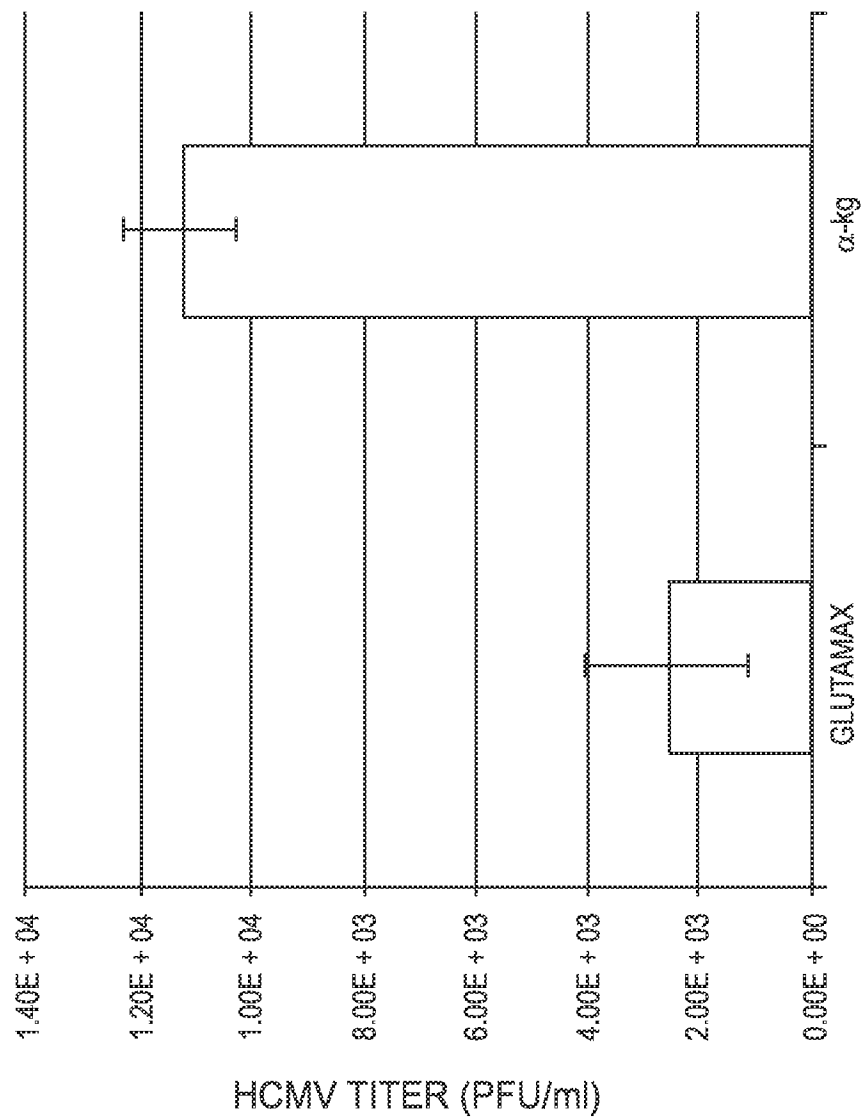


Figure 13

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2013/036878

| <b>A. CLASSIFICATION OF SUBJECT MATTER</b><br>IPC(8) - C12N 7/00 (2013.01)<br>USPC - 424/204.1<br>According to International Patent Classification (IPC) or to both national classification and IPC                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |                                                                                                                                                                               |                                                                                                     |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------|
| <b>B. FIELDS SEARCHED</b><br>Minimum documentation searched (classification system followed by classification symbols)<br>IPC(8): A61K 39/245; C12N 7/06, 15/09, 15/38 (2013.01)<br>USPC: 424/204.1; 435/4, 5, 235.1<br>Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched<br>CPC Class/Subclass(es): A61K 2039/525; C12N 7/00, 7/06, 2710/16151<br>Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)<br>Patbase, Google Patents, Google, Pubmed                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |                                                                                                                                                                               |                                                                                                     |
| <b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                                                                                                                                                               |                                                                                                     |
| Category*                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | Citation of document, with indication, where appropriate, of the relevant passages                                                                                            | Relevant to claim No.                                                                               |
| X                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | CHAMBERS et al. 'Glutamine Metabolism Is Essential for Human Cytomegalovirus Infection.' Journal of Virology. Vol. 84, No. 4: Pages 1867-1873. February 2010. entire document | 1-3                                                                                                 |
| A                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | WO 1992/20786 A1(SANGAR et al) 26 November 1992 (26.11.1992) entire document                                                                                                  | 1-3                                                                                                 |
| A                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | US 2010/0184190 A1 (TRUONG-LE) 22 July 2010 (22.07.2010) entire document                                                                                                      | 1-3                                                                                                 |
| <input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |                                                                                                                                                                               |                                                                                                     |
| * Special categories of cited documents:<br>"A" document defining the general state of the art which is not considered to be of particular relevance<br>"E" earlier application or patent but published on or after the international filing date<br>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)<br>"O" document referring to an oral disclosure, use, exhibition or other means<br>"P" document published prior to the international filing date but later than the priority date claimed<br>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention<br>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone<br>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art<br>"&" document member of the same patent family |                                                                                                                                                                               |                                                                                                     |
| Date of the actual completion of the international search<br>29 July 2013                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                                                                                                                               | Date of mailing of the international search report<br><b>09 AUG 2013</b>                            |
| Name and mailing address of the ISA/US<br>Mail Stop PCT, Attn: ISA/US, Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-3201                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                                                                                                                                                               | Authorized officer:<br>Blaine R. Copenheaver<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2013/036878

## Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 4-111  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.