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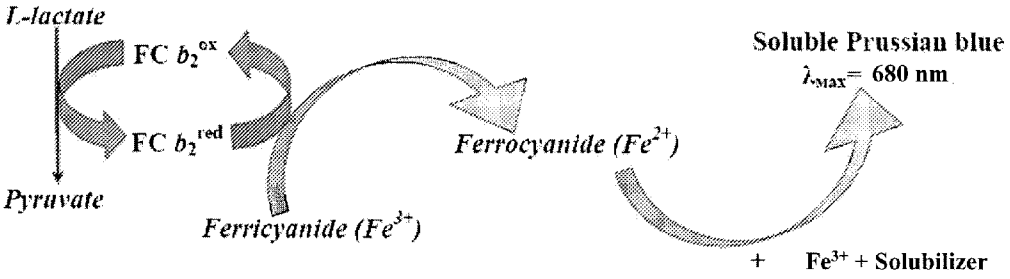
(54) **Title:** FLAVOCYTOCHROME B_2-BASED ENZYMATIC COMPOSITION, METHOD AND KIT FOR L-LACTATE

Figure 1



(57) **Abstract:** The present invention describes compositions, methods and kits for the detection of L-lactate or measurement of the concentration of L-lactate in a sample; particularly in a solution. The composition used in the methods and kits comprises cytochrome c-oxidoreductase (flavocytochrome b_2 ("FC b_2")); ferricyanide ($(Fe(CN)_6)^{3-}$); a quantity of iron ions (Fe^{3+}); and a solubilizer. The invention utilizes the catalytic properties of FC b_2 and the formation of soluble Prussian Blue to detect L-lactate.

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FLAVOCYTOCHROME b_2-BASED ENZYMATIC COMPOSITION, METHOD AND KIT FOR L-LACTATE

FIELD OF INVENTION

This invention relates to compositions, methods and kits for the detection of L-lactate.

BACKGROUND

All publications herein are incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference. The following description includes information that may be useful in understanding the present invention. It is not an admission that any of the information provided herein is prior art or relevant to the presently claimed invention, or that any publication specifically or implicitly referenced is prior art.

The detection and measurement of L-lactate is important in various fields. In the food and beverage industries, as well as clinical diagnostics, there is a need for highly-selective, sensitive, rapid, and reliable methods of determining the presence of key ingredients or metabolites which determine the quality of the product or serve as markers for diseases or the physiological state of humans. L-lactate is one of these metabolites. For example, L-lactate determination is important in the quality control of alcoholic beverages, milk products, and other foods. Also, analysis of lactate levels in blood is important in clinical diagnosis of hypoxia, lactic acidosis, and drug toxicity tests, hyperlactemia in diabetic and liver diseases, sepsis, and thiamine deficit. It is also measured when monitoring the performance of athletes and developing optimum training regimens.

In humans, pyruvate is converted to lactate by lactate dehydrogenase ("LDH").

$$\text{pyruvate} \xrightarrow{\text{LDH}} \text{lactate}$$

In yeast, L-lactate is converted to pyruvate by L-lactate:cytochrome c-oxidoreductase (EC 1.1.2.3; flavocytochrome b_2) ("FC b_2").

$$\text{L-lactate} \xrightarrow{\text{FC } b_2} \text{pyruvate} + 2H^+ + 2e^-$$

The determination of lactate content is typically based on the enzymatic oxidation of L-lactate to pyruvate. Traditionally, the enzymatic methods are based on NAD^+-dependent LDH isolated from animal muscles or heart, or on bacterial lactate oxidase used in a chromogenic system. Many other methods have been proposed as well; for example, spectrophotometry, fluorimetry, pH potentiometric measurements, and amperometric biosensors based on O_2 and H_2O_2 electrodes.

The actual lactate content is determined by the spectrophotometric detection of NADH or a colorimetric assay of H_2O_2. However, all of the current methods of lactate measurement have drawbacks. For the LDH-based method, the equilibrium constant is far on the side of L-lactate, which requires the addition of toxic compounds or other enzymes to shift the equilibrium to the pyruvate product side. In addition, current methods are not very sensitive or selective, are time-consuming, and require exogenous co-enzymes, qualified personnel, expensive equipment, and labor-intensive procedures such as filtration, chromatography, and deproteinization. The LDH-based method also requires monitoring of the reaction product (NADH) in ultraviolet spectra.

As such there exists a need in the art for improved and/or alternative methods of detecting and measuring L-lactate.

SUMMARY OF THE INVENTION

The following embodiments and aspects thereof are described and illustrated in conjunction with compositions and methods which are meant to be exemplary and illustrative, not limiting in scope.

The invention describes a method of detecting L-lactate in a sample, comprising: providing a composition, comprising: cytochrome c-oxidoreductase (flavocytochrome b_2 ("FC b_2")), ferricyanide ($(Fe(CN)_6)^{3-}$), a quantity of iron ions (Fe^{3+}) and a solubilizer; and

contacting the composition to the sample, wherein the formation of Prussian blue indicates the presence of L-lactate.

The present invention also describes a method of measuring L-lactate content in a sample, comprising: providing a composition comprising: cytochrome c-oxidoreductase (flavocytochrome b_2 ("FC b_2")), ferricyanide ($(Fe(CN)_6)^{3-}$), a quantity of iron ions (Fe^{3+}) and a solubilizer; contacting the composition to the sample; detecting the absorbance level at a wavelength of about 600 to about 800 nm; and correlating the absorbance level to pre-established data to determine the concentration of L-lactate in the test sample. In one embodiment, the wavelength may be about 680 nm.

The sample in these methods may be a fluid. In particular embodiments, the sample may be selected from the group consisting of a beverage, a milk product, a body fluid and fluidized tissue. In a specific embodiment, the body fluid may be blood.

The present invention also describes a composition for use in the detection of L-lactate or measurement of the concentration of L-lactate in a sample, comprising: cytochrome c-oxidoreductase (flavocytochrome b_2 ("FC b_2")); ferricyanide ($(Fe(CN)_6)^{3-}$); a quantity of iron ions (Fe^{3+}); and a solubilizer. In a further embodiment, the composition may comprise a buffer.

The present invention further describes a kit for detecting or measuring L-lactate in a sample, comprising: cytochrome c-oxidoreductase (flavocytochrome b_2 ("FC b_2")); ferricyanide ($(Fe(CN)_6)^{3-}$); a quantity of iron ions (Fe^{3+}); a solubilizer; and instructions to use the FC b2, the $Fe(CN)_6)^{3-}$, the quantity of iron ions and the solubilizer to detect or measure L-Lactate in a sample. In an alternative embodiment, the FC b2, the $Fe(CN)_6)^{3-}$, the quantity of iron ions and the solubilizer may be provided in one composition.

In various embodiments, the FC b_2 used in the methods, compositions and kits may be isolated from *H. polymorpha*; the ferricyanide used in the methods, compositions and kits may be potassium ferricyanide ($K_3Fe(CN)_6$); the iron ions used in the methods, compositions and kits may be in the form of an iron salt, for example, ferric chloride ($FeCl_3$) or ferric chloride dissolved in hydrochloric acid; the solubilizer used in the methods, compositions and kits may be selected from the group consisting

of oxalic acid, citric acid, succinic acid L-aspartic acid, L-glutamic acid, malic acid, malonic acid and combinations thereof.

Other features and advantages of the invention will become apparent from the following detailed description, taken in conjunction with the accompanying drawings, which illustrate, by way of example, various features of embodiments of the invention.

BRIEF DESCRIPTION OF THE FIGURES

Exemplary embodiments are illustrated in referenced figures. It is intended that the embodiments and figures disclosed herein are to be considered illustrative rather than restrictive.

Figure 1 depicts a scheme of the reactions exploited in the enzymatic assay of L-lactate in accordance with an embodiment of the present invention.

Figure 2 depicts the detection of the maximum absorption peak of the colored product in accordance with an embodiment of the present invention.

Figure 3 depicts the evaluation of a linear range for the method to assay L-lactate in accordance with an embodiment of the present invention.

DESCRIPTION OF THE INVENTION

All references cited herein are incorporated by reference in their entirety as though fully set forth. Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Singleton *et al.*, *Dictionary of Microbiology and Molecular Biology 3rd ed.*, J. Wiley & Sons (New York, NY 2001); March, *Advanced Organic Chemistry Reactions, Mechanisms and Structure 5th ed.*, J. Wiley & Sons (New York, NY 2001); and Sambrook and Russel, *Molecular Cloning: A Laboratory Manual 3rd ed.*, Cold Spring Harbor Laboratory Press (Cold Spring Harbor, NY 2001), provide one skilled in the art with a general guide to many of the terms used in the present application.

One skilled in the art will recognize many methods and materials similar or equivalent to those described herein, which could be used in the practice of the present

invention. Indeed, the present invention is in no way limited to the methods and materials described. For purposes of the present invention, the following term is defined below.

"Solubilizer" as used herein refers to an agent that enables Prussian blue to be soluble in solution.

The inventors have discovered a new method to detect L-lactate in solution using FC b_2. FC b_2 has unique catalytic properties that make it an excellent candidate to replace NAD^+-dependent LDH or lactate oxidase in enzymatic and biosensor assays of L-lactate. These properties include absolute selectivity to the stereoconfiguration of L-lactate and non-dependence on exogenous co-enzymes. This novel method is very sensitive for L-lactate, simple to prepare, and does not require expensive equipment. In one embodiment, a simple photocolorimeter may be sufficient to carry out the inventive method.

FC b_2 can be isolated from *S. cerevisiae* (simple baker's yeast) and *H. anomala*. However, FC b_2 from *S. cerevisiae* is very labile and difficult to isolate and purify, and may thus be difficult to use in bioanalytical devices. Still, it may be useful in various embodiments of the invention. FC b_2 from the thermotolerant yeast *H. polymorpha*, however, is thermostable and can be used successfully as a biorecognition element of biosensors selective for L-lactate.

The invention is a composition, method and kit for assaying L-lactate based on the use of thermostable FC b_2 (*e.g.*, from *H. polymorpha*) and the detection of Prussian blue. Normally, Prussian blue is insoluble in solution; however, the inventors have discovered solubilizers that can be used to transform Prussian blue into soluble form in solution.

In the inventive technique, L-lactate is oxidized by FC b_2 to pyruvate. The reduced FC b_2 reacts with ferricyanide ($Fe(CN)_6^{3-}$) to yield ferrocyanide ($Fe(CN)_6^{4-}$). Ferrocyanide reacts with iron (Fe^{3+}) ions to form an intensely colored Prussian blue. See Figure 1.

Prussian blue has notoriously low solubility and could not be used directly for colorimetric assay of L-lactate in solution since Prussian blue is insoluble in water. The

inventors have added a compound that transforms Prussian blue into soluble form, which means L-lactate content can be assayed in solution, yielding a technique with many practical applications. The solubilizer may be an organic acid with chelating ability, which may allow solubilization and/or stabilization of the product. In various embodiments, the solubilizer may be oxalic acid, citric acid, succinic acid, L-aspartic acid, L-glutamic acid, malic acid, malonic acid or combinations thereof. The solubilizer may be in concentrations of from about 0.1 M to about 1 M. In particular embodiments, the solubilizer may be in concentrations of from about 0.25 M to about 0.75 M and from about 0.33 M to about 0.5 M. In various embodiments, the concentrations of oxalic acid may be about 0.1 M to about 1 M, or about 0.25 M to about 0.75 M; the concentrations of citric acid may be about 0.1 M to about 1 M, or about 0.25 M to about 0.75 M; the concentrations of succinic acid may be about 0.1 M to about 1 M, about 0.25 M to about 0.75 M, or about 0.33 M to about 0.5 M; the concentrations of L-aspartic acid may be about 0.1 M to about 1 M, or about 0.25 M to about 0.75 M; and the concentrations of L-glutamic acid may be about 0.1 M to about 1 M, or about 0.25 M to about 0.75 M. In specific embodiments, the concentrations of the oxalic acid, citric acid, succinic acid, L-aspartic acid, and L-glutamic acid may be about 0.5 M, 0.5 M, 0.33 M, 0.5 M, and 0.5 M, respectively. See Table 1.

Table 1

Solubilizer	**λ max**	**OD**	**Final pH**	**Stability, not less min**
0.5 M Oxalic acid	680	1.326	0.71	30-40
0.5 M Citric acid	690	0.932	1.03	15
0.33 M Succinic acid	680	0.566	1.66	24 hr
0.5 M L-Aspartic acid in 1 M HCl	690	0.872	0.36	30-40
0.5 M L-Glutamic acid in 1 M HCl	690	0.796	0.35	30-40
0.5 M α-Ketoglutaric acid	-	-	1.31	Unstable

One embodiment of the present invention provides for a composition for detecting L-lactate in a sample (particularly in a liquid sample) or for determining the concentration of L-lactate in a sample (particularly in a liquid sample), comprising: cytochrome c-oxidoreductase (flavocytochrome b_2 ("FC b_2")), ferricyanide ($(Fe(CN)_6)^{3-}$), a quantity of iron ions (Fe^{3+}), and a solubilizer. In a further embodiment, the composition may further comprise a buffer.

Another embodiment of the present invention provides for a method of detecting L-lactate in a sample, comprising: providing a composition, comprising: cytochrome c-oxidoreductase (flavocytochrome b_2 ("FC b_2")), ferricyanide ($(Fe(CN)_6)^{3-}$), a quantity of iron ions (Fe^{3+}) and a solubilizer; and contacting the composition to the sample, wherein the formation of Prussian blue indicates the presence of L-lactate. The composition may further comprise a buffer.

In an alternative embodiment, the FC b_2, the ferricyanide, the solubilizer, the quantity of iron ions and/or the buffer are provided separately. Furthermore, contacting the reagents with the test sample may be performed simultaneously or consecutively in any order. In a particular embodiment, the test sample is contacted with the quantity of FC b_2 and the ferricyanide before being contacted with the quantity of iron ions and the solubilizer.

The maximal absorption peak of the colored product is near λ=680 nm (Fig. 2) and the linear range is about 0.008 – 0.27 mM for L-lactate (Fig. 3). Thus, in one embodiment a detection of about λ=680 nm indicates the presence of lactate in the test sample. In another embodiment, a detection of a wavelength of at least 512 nm indicates the presence of lactate in the test sample. In other embodiments, a detection of a wavelength from about 512 nm to about 845 nm, from about 600 nm to about 800 nm, or from about 640 nm to about 720 nm indicates the presence of lactate in the test sample.

In one embodiment, the method has a minimum sensitivity level of about 3 µM of L-lactate. In another embodiment, the method has sensitivity levels of about 3 µM to about 0.333 nM of L-lactate. In another embodiment, the method has sensitivity levels of about 3 µM of L-lactate to about 0.27 mM of L-lactate.

Another embodiment of the present invention provides for a method of measuring L-lactate content in a sample, comprising: providing a composition comprising: cytochrome c-oxidoreductase (flavocytochrome b_2 ("FC b_2")), ferricyanide ($(Fe(CN)_6)^{3-}$), a quantity of iron ions (Fe^{3+}) and a solubilizer; contacting the composition to the sample; detecting the absorbance level at a wavelength of about 600 to about 800 nm; and correlating the absorbance level to pre-established data to determine the concentration of L-lactate in the test sample. (See fig. 3.) This correlation is possible because the optical density of the final reaction product is proportional to the concentration of lactate in the test sample. The composition may further comprise a buffer

In an alternative embodiment, the FC b_2, the ferricyanide, the solubilizer, the quantity of iron ions and/or the buffer are provided separately. Furthermore, contacting the reagents with the test sample may be performed simultaneously or consecutively in any order. In a particular embodiment, the test sample is contacted with the quantity of FC b_2 and the ferricyanide before being contacted with the quantity of iron ions and the solubilizer.

In a particular embodiment the absorbance level is detected at a wavelength of about 680 nm. In other embodiments, the absorbance level may be detected at wavelength of at least 512 nm, from about 512 nm to about 845 nm, from about 600 nm to about 780 nm or from about 640 nm to about 720 nm. Thus, correlating the absorbance level to pre-established data to determine the concentration of L-lactate in the test sample is made to pre-established data that was compiled at the desired wavelength.

Another embodiment of the present invention provides for a post assay composition wherein Prussian Blue is formed, comprising: pyruvate, cytochrome c-oxidoreductase ("FC b_2"), a solubilizer, and a quantity of Prussian Blue. In further embodiments, the composition may further comprise a quantity of ferricyanide, a quantity of ferrocyanide, a quantity of iron ions, a buffer or combinations thereof.

Another embodiment of the present invention provides for a post assay composition wherein Prussian Blue is not formed, comprising: a test sample, cytochrome c-oxidoreductase ("FC b_2"), ferricyanide, a solubilizer and a quantity of iron ions (Fe^{3+}). In a further embodiment, the post test composition further comprises a buffer.

The present invention is also directed to a kit to detect the presence of L-lactate in solution and a kit to determine the concentration of L-lactate. The kit is an assemblage of materials or components, including at least one of the inventive compositions. Thus, in some embodiments the kit contains cytochrome c-oxidoreductase (flavocytochrome b_2 ("FC b_2")), ferricyanide ($(Fe(CN)_6)^{3-}$), a quantity of iron ions (Fe^{3+}) and a solubilizer. In another embodiment, the kit may further comprise a buffer. In another embodiment, these reagents may be provided as one composition.

The exact nature of the components configured in the inventive kit depends on its intended purpose. For example, various embodiments are configured for the purposes of detecting L-lactate in food and beverages, in clinical diagnostics, in the monitoring of athlete performance, in development of optimum training regimens, and in other biotechnological applications. Other embodiments are configured for the purposes of determining the concentration of L-lactate in food and beverages, in clinical diagnostics, in the monitoring of athlete performance, in development of optimum training regimens, and in other biotechnological applications. In one embodiment, the kit is configured particularly for the purpose of detecting L-lactate or determining the concentration of L-lactate in mammalian subjects; particularly, human subjects.

Instructions for use may be included in the kit. "Instructions for use" typically include a tangible expression describing the technique to be employed in using the components of the kit to effect a desired outcome, such as to detect and/or quantify the concentration of L-lactate in solution. For example, the kit may include instructions to contact the FC b_2, the ferricyanide, the solubilizer, and the quantity of iron ions with a test sample, and to determine whether L-lactate is present in the test sample by detecting Prussian blue, a detection of Prussian blue being an indication that L-lactate is

present in the test sample. The instruction may also comprise instructions to quantify the concentration of L-lactate by detecting the absorption at a specific wavelength and correlating the absorption with predetermined data to determine the concentration of L-lactate in the test sample. Optionally, the kit also contains other useful components, such as, diluents, additional buffers, syringes, catheters, applicators, test tubes, pipetting or measuring tools, multiple well plates or other useful paraphernalia as will be readily recognized by those of skill in the art.

The materials or components assembled in the kit can be provided to the practitioner stored in any convenient and suitable ways that preserve their operability and utility. For example the components can be in dissolved, dehydrated, or lyophilized form; they can be provided at room, refrigerated or frozen temperatures. The components are typically contained in suitable packaging material(s). As employed herein, the phrase "packaging material" refers to one or more physical structures used to house the contents of the kit, such as inventive compositions and the like. The packaging material is constructed by well known methods, preferably to provide a sterile, contaminant-free environment. As used herein, the term "package" refers to a suitable solid matrix or material such as glass, plastic, paper, foil, and the like, capable of holding the individual kit components. Thus, for example, a package can be one or more glass vials used to contain suitable quantities of FC b_2, ferricyanide, the solubilizer, and/or the iron ions. The packaging material generally has an external label which indicates the contents and/or purpose of the kit and/or its components.

The methods and kits of the present invention may be useful for numerous applications. As such, the kits may be particularly configured for use in a specific application wherein the detection of and/or determination of the concentration of L-lactate is desired; for example, quality control of beverages, quality control of alcoholic beverages, quality control of milk products, analysis of lactate levels in blood, diagnosis of hypoxia, diagnosis of lactic acidosis, drug toxicity tests, diagnosis of hyperlactemia (*e.g.*, in diabetic patients or patients with liver disease), diagnosis of sepsis, and diagnosis of thiamine deficiency.

FC b_2 used in the methods, compositions and kits of the present invention may be isolated from yeast; for example, *S. cerevisiae*, *H. anamala* and *H. polymorpha*. One particularly useful FC b_2 is FC b_2 isolated from *H. polymorpha*.

The ferricyanide used in the methods, compositions and kits of the present invention may be a salt; for example, as potassium ferricyanide ($K_3Fe(CN)_6$). In a particular embodiment, potassium ferricyanide ($K_3Fe(CN)_6$) may be in a 0.1 mM concentration.

The solubilizer used in the methods, compositions and kits of the present invention may be an acid; however, strong mineral acids should not be used as they may destroy the Prussian Blue complex and release free HCN. The solubilizer may be an organic acid with chelating ability, which may allow solubilization and/or stabilization of the product. In various embodiments, the solubilizer may be oxalic acid, citric acid, succinic acid, L-aspartic acid, L-glutamic acid, malic acid, malonic acid or combinations thereof. The solubilizer may be in concentrations of from about 0.1 M to about 1.0 M, from about 0.25 M to about 0.75 M, or from about 0.33 M to about 0.5 M. In specific embodiments, the concentrations of the oxalic acid, citric acid, succinic acid, L-aspartic acid, and L-Glutamic acid may be 0.5 M, 0.5 M, 0.33 M, 0.5 M, and 0.5 M, respectively. See Table 1 above.

The iron ions in the methods, compositions and kits of the present invention may be a salt; for example, ferric chloride ($FeCl_3$); particularly, ferric chloride ($FeCl_3$) dissolved in hydrochloric acid (HCl). In a particular embodiment, the iron ions may be provided as 20 mM $FeCl_3$ in 20 mM of HCl.

The buffer used in the methods, compositions and kits of the present invention may be a phosphate buffer. The phosphate buffer may be at a 25 mM concentration and may be at a pH of 7.7.

The methods, compositions and kits for detecting L-lactate or for determining the concentration of L-lactate may be useful in a variety of applications; for example, quality control of beverages, quality control of alcoholic beverages, quality control of milk products, analysis of lactate levels in blood, diagnosis of hypoxia, diagnosis of lactic

acidosis, drug toxicity tests, diagnosis of hyperlactemia (*e.g.*, in diabetic patients or patients with liver disease), diagnosis of sepsis, and diagnosis of thiamine deficiency.

As such, in one embodiment, the test sample is a quantity of a beverage. In another embodiment, the test sample is a quantity of an alcoholic beverage. In another embodiment, the test sample is a quantity of a milk product. In another embodiment, the test sample is a quantity of blood. In still another embodiment, the test sample is a quantity of a body fluid for fluidized tissue (*e.g.*, CSF (spinal fluid), urine, sweat, saliva, tears, pulmonary secretions, breast aspirate, prostate fluid, seminal fluid, stool, cervical scraping, cysts, amniotic fluid, intraocular fluid, mucous, moisture in breath, animal tissue, cell lysates, tumor tissue, hair, skin, buccal scrapings, nails, bone marrow, cartilage, prions, bone powder, ear wax, etc.).

Various embodiments of the invention are described above in the Detailed Description. While these descriptions directly describe the above embodiments, it is understood that those skilled in the art may conceive modifications and/or variations to the specific embodiments shown and described herein. Any such modifications or variations that fall within the purview of this description are intended to be included therein as well. Unless specifically noted, it is the intention of the inventors that the words and phrases in the specification and claims be given the ordinary and accustomed meanings to those of ordinary skill in the applicable art(s).

The foregoing description of various embodiments of the invention known to the applicant at this time of filing the application has been presented and is intended for the purposes of illustration and description. The present description is not intended to be exhaustive nor limit the invention to the precise form disclosed and many modifications and variations are possible in the light of the above teachings. The embodiments described serve to explain the principles of the invention and its practical application and to enable others skilled in the art to utilize the invention in various embodiments and with various modifications as are suited to the particular use contemplated. Therefore, it is intended that the invention not be limited to the particular embodiments disclosed for carrying out the invention.

While particular embodiments of the present invention have been shown and described, it will be obvious to those skilled in the art that, based upon the teachings herein, changes and modifications may be made without departing from this invention and its broader aspects and, therefore, the appended claims are to encompass within their scope all such changes and modifications as are within the true spirit and scope of this invention. It will be understood by those within the art that, in general, terms used herein are generally intended as "open" terms (*e.g.*, the term "including" should be interpreted as "including but not limited to," the term "having" should be interpreted as "having at least," the term "includes" should be interpreted as "includes but is not limited to," etc.).

WHAT IS CLAIMED IS:

1. A method of detecting L-lactate in a sample, comprising:
providing a composition, comprising:
cytochrome c-oxidoreductase (flavocytochrome b_2 ("FC b_2")),
ferricyanide ($(Fe(CN)_6)^{3-}$), a quantity of iron ions (Fe^{3+}) and a solubilizer; and
contacting the composition to the sample,
wherein the formation of Prussian blue indicates the presence of L-lactate.
2. The method of claim 1, wherein the FC b_2 is isolated from *H. polymorpha*.
3. The method of claim 1, wherein the ferricyanide is potassium ferricyanide ($K_3Fe(CN)_6$).
4. The method of claim 1, wherein the iron ions are in the form of an iron salt.
5. The method of claim 4, wherein the iron salt is ferric chloride ($FeCl_3$).
6. The method of claim 5, wherein the ferric chloride is dissolved in hydrochloric acid.
7. The method of claim 1, wherein the solubilizer is selected from the group consisting of oxalic acid, citric acid, succinic acid L-aspartic acid, L-glutamic acid, malic acid, malonic acid and combinations thereof.
8. The method of claim 1, wherein the sample is a fluid.
9. The method of claim 1, wherein the sample is selected from the group consisting of a beverage, a milk product, a body fluid and fluidized tissue.
10. The method of claim 9, wherein the body fluid is blood.
11. A composition for use in the detection of L-lactate and/or measurement of the concentration of L-lactate in a sample, comprising:
cytochrome c-oxidoreductase (flavocytochrome b_2 ("FC b_2"));
ferricyanide ($(Fe(CN)_6)^{3-}$);
a quantity of iron ions (Fe^{3+}); and
a solubilizer.
12. The composition of claim 11, further comprising a buffer.

13. The composition of claim 11, wherein the FC b_2 is isolated from *H. polymorpha*.
14. The composition of claim 11, wherein ferricyanide is potassium ferricyanide ($K_3Fe(CN)_6$).
15. The composition of claim 11, wherein the iron ions are in the form of an iron salt.
16. The composition of claim 15, wherein the iron salt is ferric chloride ($FeCl_3$).
17. The composition of claim 16, wherein the ferric chloride is dissolved in hydrochloric acid.
18. The composition of claim 11, wherein the solubilizer is selected from the group consisting of oxalic acid, citric acid, succinic acid L-aspartic acid, L-glutamic acid, malic acid, malonic acid and combinations thereof.
19. A method of measuring L-lactate content in a sample, comprising:

providing a composition comprising:

cytochrome c-oxidoreductase (flavocytochrome b_2 ("FC b_2")),

ferricyanide ($(Fe(CN)_6)^{3-}$), a quantity of iron ions (Fe^{3+}) and a solubilizer;

contacting the composition to the sample;

detecting the absorbance level at a wavelength of about 600 to about 800 nm; and

correlating the absorbance level to pre-established data to determine the concentration of L-lactate in the test sample.

20. The method of claim 19, wherein the FC b_2 is isolated from *H. polymorpha*.
21. The method of claim 19, wherein ferricyanide is potassium ferricyanide ($K_3Fe(CN)_6$).
22. The method of claim 19, wherein the iron ions are in the form of an iron salt.
23. The method of claim 22, wherein the iron salt is ferric chloride ($FeCl_3$).
24. The method of claim 23, wherein the ferric chloride is dissolved in hydrochloric acid.
25. The method of claim 19, wherein the solubilizer is selected from the group consisting of oxalic acid, citric acid, succinic acid L-aspartic acid, L-glutamic acid, malic acid, malonic acid and combinations thereof.
26. The method of claim 19, wherein the wavelength is about 680 nm.

27. The method of claim 19, wherein the sample is a fluid.

28. The method of claim 19, wherein the sample is selected from the group consisting of a beverage, a milk product, a body fluid, and fluidized tissue.

29. The method of claim 28, wherein the body fluid is blood.

30. A kit for detecting or measuring L-lactate in a sample, comprising:

cytochrome c-oxidoreductase (flavocytochrome b_2 ("FC b_2"));

ferricyanide ($(Fe(CN)_6)^{3-}$);

a quantity of iron ions (Fe^{3+});

a solubilizer; and

instructions to use the FC b2, the $Fe(CN)_6)^{3-}$, the quantity of iron ions and the solubilizer to detect or measure L-Lactate in a sample.

31. The kit of claim 30, wherein the FC b_2 is isolated from *H. polymorpha*.

32. The kit of claim 30, wherein ferricyanide is potassium ferricyanide ($K_3Fe(CN)_6$).

33. The kit of claim 30, wherein the iron ions are in the form of an iron salt.

34. The kit of claim 33, wherein the iron salt is ferric chloride ($FeCl_3$).

35. The kit of claim 30, wherein the solubilizer is selected from the group consisting of oxalic acid, citric acid, succinic acid L-aspartic acid, L-glutamic acid, malic acid, malonic acid and combinations thereof.

36. The kit of claim 30, wherein the FC b2, the $Fe(CN)_6)^{3-}$, the quantity of iron ions and the solubilizer are provided in one composition.

Figure 1

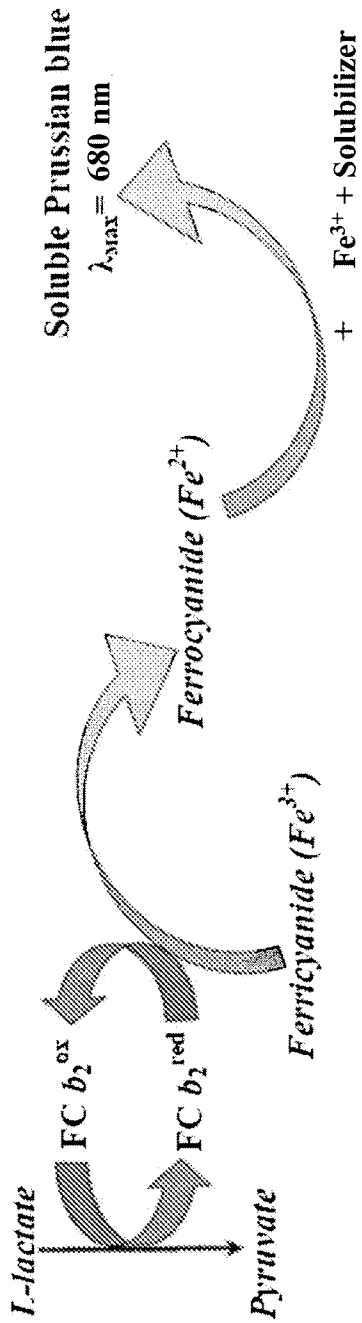

L-lactate
FC b_2^{ox}
FC b_2^{red}
Pyruvate
Ferricyanide (Fe^{3+})
Ferrocyanide (Fe^{2+})
Soluble Prussian blue
λ_{max} = 680 nm
+ Fe^{3+} + Solubilizer

Figure 3

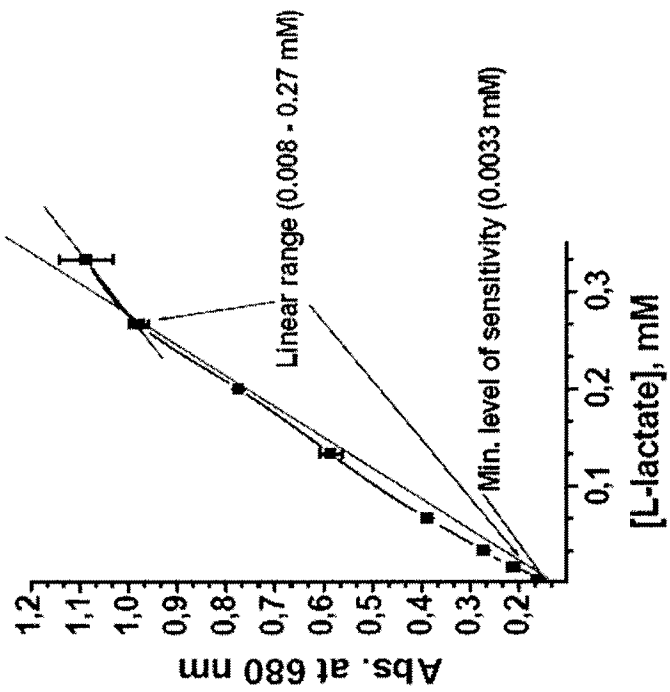
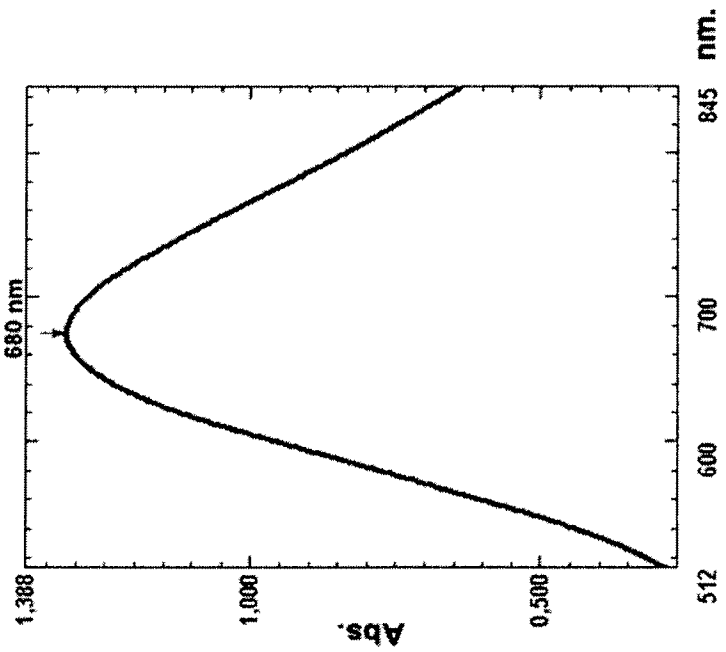


Figure 2



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 08/69637

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12Q 1/26, 1/52 (2008.04)
USPC - 435/16, 25
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC: 435/16, 25

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 435/16, 25, 7.1, 189-190; 424/677; 426/582
(text search)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST(PGPB,USPT,USOC,EPAB,JPAB); Google; PubMed
Search terms: l-lactate, flavocytochrome, fc b2, ferricyanide, prussian blue, citric acid, soluble, solublize, iron, polymorpha

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	GAIDA, G.Z. et al. New method for detection of enzymatic activity of flavocytochrome b2 in electrophoregrams. Prikl. Biokhim. Mikrobiol. March-April 2003 (03-04.2003), Vol. 39, No. 2, pages 249-252; abstract	1-36
Y	US 5,434,055 A (JERNIGAN, W.) 18 July 1995 (18.07.1995) claim 11; col 1, ln 25-29, 39-52; col 3, ln 18-28, 45-53; col 4, ln 30-32; col 6, ln 58-60; col 7, ln 9-30	1-36
Y	RAHMAN, N. et al. Kinetic spectrophotometric analysis of pantoprazole in commercial dosage forms. Anal. Sci. July 2006 (07.2006), Vol. 22, No. 7, pages 983-988; abstract	19-29

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
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"O" document referring to an oral disclosure, use, exhibition or other means
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"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
"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
"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
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 September 2008 (21.09.2008)	25 SEP 2008
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

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