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(54) Title: ARTICLES OF MANUFACTURE AND METHODS FOR STAINING BIOMOLECULES

(57) Abstract

Biomolecules contained in gels or on membranes can be stained or labeled, without direct contact with toxic staining solutions, by applying to the gel or membrane an article of manufacture comprising a flexible backing with a coating that contains a stain or label. During the application, the stain or label is transferred from the backing to the biomolecules, marking them. The volume of the staining solution in this procedure is minimized, and environmental hazards are reduced. Furthermore, ease of manufacture reduces the cost of the article.

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ARTICLES OF MANUFACTURE AND METHODS FOR STAINING BIOMOLECULES

Background of the Invention

Electrophoresis techniques have become principal tools for characterizing biomolecules. The method is based on the fact that macromolecules such as DNA, RNA and proteins possess a charge and can therefore move in an electric field through sieving materials such as agarose or polyacrylamide. The application of electrophoretic techniques has required the development of chemical indicators for use in visualizing separated macromolecules. One such indicator used to visualize DNA is ethidium bromide (EtBr). The disadvantage of this widely used stain is that it is a potent mutagen. The potential personal hazard of directly contacting such a solution and the environmental hazard of pouring it or other hazardous chemicals down the drain has resulted in a need for a better and safer method of preparing, using and disposing of such toxic solutions.

A procedure using entrapped stain in gels provides a vast improvement for the containment of dyes in gels (Chirikjian and Collier, U.S. Patent No. 5,776,684). However, these gels have several disadvantages which include stability, storage, sensitivity to cold temperatures and relative fragility. Furthermore, a lack of manufacturability increases the cost of these gels precluding their wide spread use. It is apparent, therefore, that an apparatus which economically provides a means of safely staining biomolecules is needed.

Summary of the Invention

It is therefore an object of the present invention to provide a readily manufacturable apparatus capable of safely staining or labeling biomolecules.

In accomplishing this and other objects of the invention, there is provided, in accordance with one aspect of the present invention, an article of

manufacture comprising a flexible backing and a dry coating adhered to a surface of the backing, wherein the coating comprises an indicator that stains or labels a biomolecule. In preferred embodiments of the present invention, the backing promotes good contact with a gel containing a biomolecule, is impermeable, allows the coating solution to be evenly spread and dried and is easily dispensable. In other preferred embodiments, the coating solution is selected from the group consisting of starch, glue, gum, flour, agarose or polyacrylamide, or mixtures of two or more of these. In still other preferred embodiments, the indicator is selected from the group consisting of stains, dyes or labeled probes.

In accordance with another aspect of the present invention, there is provided a method for staining or labeling biomolecules, comprising applying an article of manufacture comprising a flexible backing and a dry coating adhered to a surface of the backing, wherein the coating comprises an indicator that stains or labels a biomolecule, to a gel or support containing the biomolecules for a sufficient period of time to allow diffusion to the biomolecules.

In accordance with yet another aspect of the present invention, there is provided a kit suitable for use in a method for staining or labeling biomolecules, the kit comprising (A) an article of manufacture comprising a flexible backing and a dry coating adhered to a surface of the backing, wherein the coating comprises an indicator that stains or labels a biomolecule; (B) reagents to effect staining or labeling of the biomolecules; and (C) instruments to effect staining or labeling of the biomolecules.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

Brief Description of the Drawings

Figure 1. Demonstration of EtBr staining of DNA using two types coated backings. Figure 1(a) shows an agarose gel stained with a backing comprising White Uncoated Paper #100. Figure 1(b) shows an agarose gel stained with a backing comprising Prevail Paper #125. The gels were stained for 2-3 minutes and then placed on a Short Wave UV Transilluminator for visualization of the DNA bands and for obtaining photographs.

Detailed Description of the Preferred Embodiments

The present invention provides an article of manufacture and methods for safely staining or labeling biomolecules. The stable, resilient and pliable article can be used repeatedly in a method for staining biomolecules in gels and membranes without directly contacting toxic staining solutions and without handling delicate gels which contain such stains. The process eliminates the environmental hazard resulting from the disposal of toxic stains since the backing with the stain can be disposed of in a solid chemical waste container. In addition, the process reduces the amount of stain to be used and eliminates the personal hazards associated with staining biomolecules since the operator will no longer have direct contact with the stain itself. Furthermore, the article is easily manufactured which reduces the cost of the article and ensures its accessibility in the marketplace.

In accordance with one aspect of the present invention, an article of manufacture is provided which comprises a flexible backing and a dry coating adhered to a surface of the backing, wherein the coating comprises an indicator that stains or labels a biomolecule. In preferred embodiments of the present invention, the backing is flexible, promotes good contact with a support or gel containing a biomolecule, is impermeable and allows the coating solution to be evenly spread and dried. The backing can be composed of cellulose-based compounds, such as various papers, for example wall paper, cloth or vinyl, 3M Whatman™ paper, vinyl paper, cloth paper, prepasted paper, to name a few. The backing can be any wood, preferably light and strong as Balsam of about 1/16-1/8 of an inch in thickness, any glass such as borosilicate of about 50 mm,

any cloth, cotton, rayon, tafetta, mesh, wool, synthetic polymers such as nylon, acetate, mixtures of cotton and acetate, polypropylene or polyethylene, polycarbonate, acrylic, cellophane acetate, Gelbond (FMC Bioproducts, Maine) to name a few, and any sponge. In another embodiment, the backing is comprised of two layers, consisting of a permeable layer and an impermeable layer.

In a preferred embodiment of the present invention, the coating solution comprises an adhering solution and an indicator capable of staining or labeling a biomolecule. Any solution which adheres to the backing and is preferably amenable to drying and rewetting, and does not degrade the biomolecule to be stained or the gel or support which retains the biomolecule to be stained can be utilized. Most preferred are adhering solutions comprised of modified starches and their derivatives. Starch solutions can be modified in a variety of ways, including digestion with amylase. Other examples of adhering solutions include, but are not limited to, water-based glues, flour, agarose, polyacrylamide and its derivatives, and any mixture there of. Examples of glues include, but are not limited to, wall-paper glue, paper glue and multiple function glue. Gums, such as locust bean gum, also can be used. Adhering solutions containing silica or diatomaceous earth also are advantageous.

In preferred embodiments, one or more indicators are entrapped in the adhering solution. In one embodiment, the desired indicator is mixed with the coating solution at varying amounts depending on the sensitivity of the stain. The indicator can be in the form of a solution. For example, the indicator can be dissolved in water or a buffer. Suitable buffers can comprise mixtures of aqueous and organic solutions or comprise only organic solutions, such as isopropanol for ethidium bromide. Alternatively, the indicator can be mixed into the coating solution as a powder. In another embodiment, the stain is spread onto a backing onto which a coating solution has been dried and which is capable of being rewetted again and redried.

A variety of indicators can be utilized individually or in combination in the present invention to stain or label biomolecules. Preferred indicators include, but are not limited to, stains, dyes and labeled probes. Examples of acceptable stains and dyes include, but are not limited to, ethidium bromide,

YoYo (Molecular Probes, Inc., Boulder, CO), Toto (Molecular Probes, inc. Boulder, CO), Stains-All (Sigma), SYBR Green I™, SYBR Green II™, SYBR Gold™ (Molecular Probes), methylene blue, crystal violet, methyl green, pyronin y, thionin, basic blue 66, basic red 49, indoine blue, safranin O, Janus green B, nile blue, pinacyanol, basic yellow 11, Coomassie Brilliant Blue R-250, Hoechst 33258 and Hoechst 33342. In one embodiment, the release of such stains from the surface of the backing can be controlled by alterations in pH.

In another embodiment, the indicator comprises one or more probes complementary to the biomolecule to be detected. Examples of a complementary probe include, but are not limited to, a labeled synthetic oligonucleotide, an antibody and antibody fragment. Such probes can be added to the coating solution, or applied to the dried backing with the coating solution instead of, or in addition to, a stain. The probe can be labeled with a radioactive label or a non-radioactive label such as biotin, alkaline phosphatase, and horseradish peroxidase, or a chemiluminescent reagent, among others. The labeled probe can be added at a concentration of about 1 - 1000 pmol/ml, preferably about 100 pmol/ml.

In another embodiment of the present invention, additional compositions are added to the coating solution. Such compositions, for example tris acetate, polyethylene glycol and its derivatives, and triglycerol, can facilitate staining or can stabilize the stain or biomolecules. Incorporating a hybridization buffer into the coating solution is especially useful when the indicator comprises a labeled probe.

The coating solution with an indicator can be spread onto one or both sides of the backing. Alternatively, the backing can be spread with the coating solution, dried, and then coated with an indicator solution. Coating and indicator solutions can be applied onto the backing by rolling them with a sponge roller or automated roller, or by brushing or spraying them onto the backing. The coated backing can be air dried, oven dried, or dried in a microwave oven.

In accordance with another aspect of the present invention, a method is provided for staining biomolecules, comprising applying an article of

manufacture comprising a flexible backing and a dry coating adhered to a surface of the backing, wherein the coating comprises an indicator that stains or labels a biomolecule, to a gel or support containing the biomolecules for a sufficient period of time to allow diffusion to the biomolecules. Usually, about 0.5 minute or longer of direct contact is required to allow the indicator to diffuse to the biomolecules. Examples of biomolecules include, but are not limited to, DNA, RNA and proteins.

In one embodiment, the present invention is used to stain or label biomolecules contained in gels. A gel containing biomolecules can be made of any sieving material such as agarose, starch, polyacrylamide synthetic matrices, or various blends of matrices. After the electrophoretic gel is stained, the backing is peeled off, and can be disposed of in the solid chemical waste, or reused. A fresh application of the desired stain may be necessary upon reuse. This process prevents direct contact with toxic staining solutions, minimizes the volume of the staining solution utilized and saves the environment from any hazards which such a staining solution may produce.

In another embodiment, the present invention is used to label biomolecules immobilized on a support. Hybridization assays, such as Southern, northern and western assays, utilize labeled probes to detect specific DNA, RNA or protein species immobilized onto membranes, such as nylon or nitrocellulose. Such immobilized biomolecules may be detected using the method described above. Instead of, or in addition to a stain, a probe, for example a labeled synthetic oligonucleotide complementary to the biomolecule to be detected, is added to the coating solution, or applied to the dried backing with the coating solution. As discussed above, the oligonucleotide can be labeled with a radioactive label or a non-radioactive label such as biotin, alkaline phosphatase, horseradish peroxidase or a chemiluminescent reagent among others. After transfer of the electrophoresed DNA onto a membrane, the backing containing the labeled probe is applied to the membrane such that the probe is in direct contact with the support containing the biomolecules to be detected and even transfer of the probe from the backing to the biomolecules is effected. The backing and the support are left in contact for several minutes up to eighteen hours or overnight. Detection of complementary binding between

the labeled probe and the immobilized biomolecule may be carried out by methods known in the art.

In accordance with yet another aspect of the present invention, the present invention provides a kit suitable for use in a method for staining or labeling biomolecules, the kit comprising an article of manufacture comprising a flexible backing and a dry coating adhered to a surface of the backing, wherein the coating comprises an indicator that stains or labels a biomolecule, reagents to effect staining or labeling of the biomolecules, and instruments to effect staining or labeling of the biomolecules. The kit can be an educational or research kit, whereby other reagents for the preparation of the separation gels are included, as well as other reagents which facilitate detecting stained biomolecules, for example buffers, glycerol solutions or enzymes.

Instruments which facilitate staining or detecting biomolecules can be included in the kit. For example, a roller which can be used to promote effective contact between the backing and the gel or support containing the biomolecules can be included. Similarly, a cassette into which the backing and the gel or support are placed and which promotes effective contact between the two can be included in the kit. Additionally, a spray applicator which can be used to apply reagents effecting staining can be included.

A kit for staining biomolecules may be packaged in a variety of ways. When the kit contains a backing with a coating solution comprising ethidium bromide for staining DNA or RNA, the coated backing must be stored such that it is not exposed to light, since ethidium bromide is light sensitive. A preferred package for this embodiment is a foil pouch. It is contemplated that a plurality of backing strips can be stored in a roll such that the desired size of backing can be cut away either using scissors or using a sharp serrated metal edge adhered to the box in which the roll is stored. Additionally, the use of perforated paper can simplify the dispensing process. In these forms, it is preferable that the coated backing be covered with a liner which when peeled away exposes the coating just prior to use so that the backings in the roll do not adhere to each other. Such a liner can be made from paper or other release liner. Alternatively, the backing can be stacked in sheets, folded and stored in a dispenser for easy removal of one sheet at a time. In another embodiment, the coating solution can

be supplied in a separate container for application onto the backing when needed. The coating solution can be supplied with or without stain, and the staining solution or powder can be provided in yet another container.

Inasmuch as many changes could be made in the above constructions, and many apparently different embodiments of the invention could be made without departing from the scope thereof, it is intended that all matters contained in the above description shall be interpreted as illustrative and not in a limiting sense.

Finally, singular usage herein should be interpreted as denoting "one or more." For example, the statement "an indicator can be spread onto one or both sides of the backing" is meant to include the application of one or more indicators, unless otherwise specified.

Described below are examples of the present invention which are provided only for illustrative purposes, and not to limit the scope of the present invention.

Example 1. ***Preparation of an Article for Staining DNA Molecules***

An adhering solution is prepared by forming a 10% solution of modified starch using warm, distilled water. An appropriate amount of ethidium bromide is then added to the adhering solution, preferably to a final concentration of between 0.05 mg/ml and 0.2 mg/ml. The resulting solution is spread over one side of a cellulose-based paper using a sponge roller and allowed to dry. After the coating solution has dried, the paper may be rolled up and stored until use.

Example 2. *Method for Staining DNA Molecules*

Two restriction digests were performed on a plasmid DNA sample. Each digest utilized approximately 1.5 μg of DNA. The digested products were fractionated, along with a λ-Hind-III molecular ladder, on a 0.8% UltraSpec Agarose™ (EDVOTEK cat. #605, Edvotek, West Bethesda, Maryland) in 1X TAE electrophoresis buffer (20 mM Tris, 6 mM sodium acetate, 1 mM Na_2EDTA, pH7.8) at 30 ml per 7x7 cm gel bed. The gels were run at 120 volts for 45 minutes and terminated when the tracking dye had migrated 4.5 cm from the wells. An adhering solution of 50% modified starch was prepared as described above. Ethidium bromide was added to a final concentration of 0.0625 mg/ml. The resulting coating solution was spread onto two types of paper backing, White Uncoated Paper #100 and Prevail Paper #125, using a sponge roller and allowed to air dry in the dark. Approximately 0.5 ml of the coating solution was applied to each backing. Following fractionation, the gels were inverted, and the coated backing was placed on the gel such that the ethidium bromide was in contact with the gel containing the DNA. The gels were stained for 2-3 minutes and then placed on a Short Wave Ultraviolet Transilluminator for visualization of the DNA bands. The results in Figure 1 show that the present invention provides a safe and effective means of staining DNA molecules contained in gels. Figure 1(a) is a photograph of a gel stained with the coated backing comprising White Uncoated Paper #100. Figure 1(b) is a photograph of a gel stained with the coated backing comprising Prevail Paper #125.

What is claimed is

1. An article of manufacture comprising a flexible backing and a dry coating adhered to a surface of said backing, wherein said coating comprises an indicator that stains a biomolecule.

2. An article of manufacture according to claim 1, wherein said backing is impermeable.

3. An article of manufacture according to claim 1, wherein said backing comprises a cellulose-based paper.

4. An article of manufacture according to claim 1, wherein said backing comprises a synthetic polymer.

5. An article of manufacture according to claim 1, wherein said coating is selected from the group consisting of starch, glue, gum, flour, agarose or polyacrylamide, or mixtures of two or more of these.

6. An article of manufacture according to claim 1, wherein said indicator is ethidium bromide.

7. An article of manufacture according to claim 1, wherein said indicator is selected from a group of stains consisting of YoYo (Molecular Probes, Inc., Boulder, CO), Toto (Molecular Probes, inc. Boulder, CO), Stains-All (Sigma), SYBR Green I™, SYBR Green II™, SYBR Gold™ (Molecular Probes), methylene blue, crystal violet, methyl green, pyronin y, thionin, basic blue 66, basic red 49, indoine blue, safranin O, Janus green B, nile blue, pinacyanol, basic yellow 11, Coomassie Brilliant Blue R-250, Hoechst 33258, Hoechst 33342, or mixtures of two or more of these.

8. An article of manufacture comprising a flexible backing and a dry coating adhered to a surface of said backing, wherein said coating comprises an indicator that labels a biomolecule.

9. An article of manufacture according to claim 8, wherein said backing is impermeable.

10. An article of manufacture according to claim 8, wherein said backing comprises a cellulose-based paper.

11. An article of manufacture according to claim 8, wherein said backing comprises a synthetic polymer.

12. An article of manufacture according to claim 8, wherein said coating is selected from the group consisting of starch, glue, gum, flour, agarose or polyacrylamide, or mixtures of two or more of these.

13. An article of manufacture according to claim 8, wherein said indicator is a labeled oligonucleotide.

14. An article of manufacture according to claim 13, wherein said oligonucleotide comprises a radioactive label.

15. An article of manufacture according to claim 13, wherein said oligonucleotide comprises a non-radioactive label.

16. An article of manufacture according to claim 8, wherein said indicator is a labeled antibody or antibody fragment.

17. An article of manufacture according to claim 16, wherein said antibody or antibody fragment comprises a radioactive label.

18. An article of manufacture according to claim 16, wherein said antibody or antibody fragment comprises a non-radioactive label.

19. A method for staining biomolecules, comprising applying the article of claim 1 to a gel or support containing said biomolecules for a sufficient period of time to allow diffusion to said biomolecules.

20. The method of claim 19, further comprising detecting said stained biomolecules by removing said backing from said gel or support and utilizing an appropriate detection device.

21. The method of claim 19, wherein said indicator is ethidium bromide.

22. The method of claim 19, wherein said indicator is selected from a group of stains consisting of YoYo (Molecular Probes, Inc., Boulder, CO), Toto (Molecular Probes, inc. Boulder, CO), Stains-All (Sigma), SYBR Green I™, SYBR Green II™, SYBR Gold™ (Molecular Probes), methylene blue, crystal violet, methyl green, pyronin y, thionin, basic blue 66, basic red 49, indoine blue, safranin O, Janus green B, nile blue, pinacyanol, basic yellow 11, Coomassie Brilliant Blue R-250, Hoechst 33258, Hoechst 33342, or mixtures of two or more of these.

23. A method for labeling biomolecules, comprising applying the article of claim 8 to a gel or support containing said biomolecules for a sufficient period of time to allow diffusion to said biomolecules.

24. The method of claim 23, further comprising detecting said labeled biomolecules by removing said backing from said gel or support and utilizing an appropriate detection device.

25. The method of claim 23, wherein said indicator is a labeled oligonucleotide.

26. The method of claim 25, wherein said oligonucleotide comprises a radioactive label.

27. The method of claim 25, wherein said oligonucleotide comprises a non-radioactive label.

28. The method of claim 23, wherein said indicator is a labeled antibody or antibody fragment.

29. The method of claim 28, wherein said antibody or antibody fragment comprises a radioactive label.

30. The method of claim 28, wherein said antibody or antibody fragment comprises a non-radioactive label.

31. A kit for staining biomolecules, comprising:

(A) an article of manufacture comprising a flexible backing and a dry coating adhered to a surface of said backing, wherein said coating comprises an indicator that stains a biomolecule;

(B) reagents to effect staining of said biomolecules; and

(C) instruments to effect staining of said biomolecules.

32. A kit for labeling biomolecules, comprising:

(A) an article of manufacture comprising a flexible backing and a dry coating adhered to a surface of said backing, wherein said coating comprises an indicator that labels a biomolecule; and

(B) reagents to effect labeling of said biomolecules; and

(C) instruments to effect labeling of said biomolecules.