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(54) **Titre : STABILISATION PAR POLYMERES DE SOLUTIONS CHROMOGENES**
(54) **Title: POLYMER STABILIZATION OF CHROMOGEN SOLUTIONS**

(57) **Abrégé/Abstract:**

Disclosed embodiments concern a composition comprising DAB chromogen, and/or derivative thereof, a stabilizer, and polymer capable of preventing or reducing DAB precipitation relative to a composition that does not comprise the polymer. Also disclosed herein is a method for using the disclosed composition and embodiments of a kit.

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(54) Title: POLYMER STABILIZATION OF CHROMOGEN SOLUTIONS

(57) Abstract: Disclosed embodiments concern a composition comprising DAB chromogen, and/or derivative thereof, a stabilizer, and polymer capable of preventing or reducing DAB precipitation relative to a composition that does not comprise the polymer. Also disclosed herein is a method for using the disclosed composition and embodiments of a kit.



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CLAIMS:

1. A composition for chromogenic immunohistochemistry comprising in a solution:
 - a) a solvent;
 - b) a diaminobenzidine (DAB) chromogen;
 - c) a stabilizer, selected from the group consisting of sodium metabisulfite and sodium bisulfite; and
 - d) a polyethyleneimine polymer soluble in the solvent,wherein precipitation of the DAB chromogen from the solution is reduced under accelerated aging conditions relative to a like composition not comprising the polyethyleneimine polymer.
2. The composition of claim 1, wherein the polyethyleneimine polymer is a linear or branched polyethyleneimine.
3. The composition of claim 2, wherein the linear or branched polyethyleneimine is a 4 kDa HCl salt of polyethyleneimine.
4. The composition of any one of claims 1 to 3, wherein the polyethyleneimine polymer has a number average molecular weight of between 1 kDa and 500 kDa, and/or wherein the polyethyleneimine polymer is 0.05 percent to 10 percent of the solution by weight.
5. The composition of any one of claims 1 to 4, wherein the DAB chromogen has a concentration of 0.1 mM to 100 mM.

6. The composition of any one of claims 1 to 5, further comprising an enhancer selected from the group consisting of imidazole, 2-hydroxypyridine, and combinations thereof.
7. The composition of any one of claims 1 to 6, wherein the stabilizer is sodium metabisulfite.
8. The composition of any one of claims 1 to 7, wherein the precipitation of the DAB chromogen is reduced by 10% to 100% under accelerated aging conditions relative to the composition that does not comprise the polyethyleneimine polymer, wherein the precipitation of the DAB chromogen is determined by either HPLC in an accelerated aging study at 45° C or by polymer screening using KHSO₄.
9. The composition of any one of claims 1 to 7, wherein the precipitation of the DAB chromogen is reduced under accelerated aging conditions relative to the composition that does not comprise the polyethyleneimine polymer, while under detection conditions the precipitation of the DAB chromogen is equivalent to the composition that does not comprise the polyethyleneimine polymer, wherein equivalent precipitation of the DAB chromogen includes generating no statistically significant deviation in average observed staining intensity, compared to the composition that does not comprise the polyethyleneimine polymer.
10. The composition of any one of claims 1 to 9,
wherein the composition remains stable under storage conditions so that the polyethyleneimine polymer forms a complex with the DAB chromogen so as to maintain the DAB chromogen in the solution, and

wherein the DAB precipitates under detection conditions to produce a signal suitable for chromogenic immunohistochemistry.

11. The composition of claim 10, wherein the DAB chromogen is 3,3'-diaminobenzidine and the stabilizer is sodium metabisulfite.
12. The composition of claim 11, wherein the composition comprises 1 to 15 mM 3,3'-diaminobenzidine, 0.1 to 6 mM sodium metabisulfite and 0.05% to 0.5% (w/w) polyethyleneimine.
13. A method for detecting a target in a tissue sample with a DAB chromogen, comprising:
 - contacting the tissue sample with a specific binding moiety, the specific binding moiety being specific to the target;
 - labeling the specific binding moiety with an enzyme conjugate;
 - contacting the tissue sample with a composition for chromogenic immunohistochemistry, the composition comprising in a solution a solvent, a DAB chromogen, a stabilizer, and a polyethyleneimine polymer soluble in the solvent, wherein the composition has reduced precipitation of the DAB chromogen under storage conditions but maintains precipitation of the DAB chromogen under detection conditions, and wherein the stabilizer is selected from the group consisting of sodium metabisulfite and sodium bisulfite;
 - contacting the tissue sample with an oxidant, wherein a reaction between the oxidant, the composition for chromogenic immunohistochemistry, and the enzyme conjugate causes the DAB chromogen to deposit proximally to the target;
 - contacting the tissue sample with a counterstain; and
 - detecting the target in the tissue sample by locating the DAB chromogen

wherein the enzyme of the enzyme conjugate is a peroxidase; and
wherein the oxidant is hydrogen peroxide.

14. The method of claim 13, wherein the tissue sample is a formalin-fixed, paraffin-embedded tissue sample.

15. The method of claim 13 or 14,
wherein the storage conditions refer to storing the composition for chromogenic immunohistochemistry in a sealed storage container that is not in fluid communication with other reagents, and
wherein the detection conditions refer to the presence of the oxidant and the composition for chromogenic immunohistochemistry deposited on the tissue sample and in contact with the enzyme.

16. The method of any one of claims 13 to 15, wherein the specific binding moiety is an antibody or a nucleic acid probe.

17. The method of any one of claims 13 to 16, wherein contacting the tissue sample with the composition for chromogenic immunohistochemistry comprises adding a first component solution comprising the DAB chromogen, a second component solution comprising the stabilizer, and a third component solution comprising the polyethyleneimine polymer to the tissue sample to form the composition for chromogenic immunohistochemistry while in contact with the tissue sample.