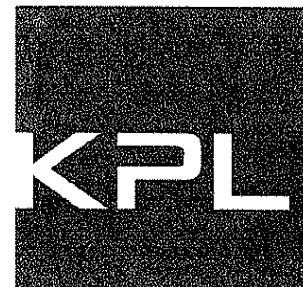


Gold Labeled Streptavidin System, 5 nm



Catalog No.
58-30-06

DESCRIPTION

Streptavidin is a 60,000 dalton protein isolated from the bacterium *Streptomyces avidinii*. The use of streptavidin rather than egg white avidin as the bridging reagent ensures that these products demonstrate sensitivity, high specificity and low background. Streptavidin is labeled with 5 nm colloidal gold using a modified proprietary method adapted from Geoghegan and Ackerman.¹

FORM/STORAGE

Gold conjugate is supplied as a liquid in 50% glycerol. Store at 2 - 8°C. Stable for a minimum of 1 year from date of receipt when stored at 2 - 8°C. May be stored at -20°C for long term stability. Gold Conjugate Dilution Buffer contains an anti-microbial agent to hinder bacterial growth.

CONTENTS

This system contains:

Catalog No. 08-30-06: 1 x 1 mL, 5 nm Gold Labeled Streptavidin

Catalog. 50-87-01: 1 x 100 mL, Gold Conjugate Dilution Buffer

APPLICATIONS

Gold labeled streptavidin is suitable for use in Light Microscopy and Electron Microscopy.

SUGGESTED WORKING DILUTIONS

Different test systems require the optimization of kit components for optimal performance. KPL recommends that serial dilutions of all reagents be evaluated to determine optimal working concentrations. Prepare working dilution in Gold Conjugate Dilution Buffer immediately before use. Storage of conjugate at a working dilution may result in performance loss.

Suggested starting dilutions are as follows:

Light Microscopy: 1:10 - 1:100

PROCEDURE

Light Microscopy

1. To deparaffinize paraffin embedded sections, place into two successive washes of xylene, or xylene substitute, for 30 minutes each.
2. Rehydrate paraffin embedded sections through decreasing concentrations of alcohol for 3 minutes each starting with 100%, 80%, 40%, ending with 20% and finally into deionized water. Other samples, i.e.

3. frozen sections, cytospin preparations, blood films, touch or squash preparations, floating or whole sections, do not require rehydration.
3. Some antigens require protease unmasking prior to incubation with the primary antibody. If needed, treat all sections for proteases with 0.1% trypsin/CaCl₂ for 20 minutes followed by a deionized water wash for 5 minutes.
4. Soak in 0.1 M Tris-HCl buffer, pH 7.6 or PBS for 10 minutes.
5. To make the Tris buffer:
 - a. Dissolve 121 g Tris in 500 mL reagent quality water.
 - b. Adjust pH to 7.6 with 2 M HCl.
 - c. QS to 1 Liter with reagent quality water to obtain a 1 M stock solution.
 - d. Dilute 1 part stock solution with 9 parts reagent quality water to prepare the working solution.
6. Block with serum block (see Related Products) for 10 minutes.
7. Incubate sample with unlabeled primary antibody, diluted in gold conjugate dilution buffer, Tris-HCl or PBS, for 15 - 20 minutes. If using a biotinylated primary antibody, proceed to step 7.
8. Wash sample for 3 x 3 minutes with 300 mM TBS (100 mM Tris/ 300 mM NaCl).
9. Incubate sample with biotinylated antibody, diluted in gold conjugate dilution buffer, Tris-HCl or PBS, for 15 - 20 minutes.
10. Wash sample for 3 x 3 minutes with 300 mM TBS (100 mM Tris/ 300 mM NaCl).
11. Incubate sample with the gold conjugate diluted in the Gold Conjugate Dilution Buffer for 15 minutes.
12. Wash sample for 3 x 3 minutes with 300 mM TBS.
13. Rinse with deionized water for 1 - 2 minutes.
14. For enhancement of signal, react sample with KPL's Silver Enhancer Kit for Microscopy Applications (see Related Products).
15. Counterstain, if desired, can be accomplished with Eosin, Contrast Green or other appropriate counterstain (see Related Products) for 30 - 60 seconds.
16. Rinse with deionized water for 1 minute.

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Catalog No.

58-30-06

17. Dehydrate through graded ethanol for 3 minutes each in 20%, 40%, 80% and ending in 100%. **NOTE:** Floating sections or whole mounts may be fixed to slides by drying under low heat followed by a 1 minute rinse in 95% ethanol.
18. Place the sample into two washes of xylene, or a xylene substitute, for 1 minute each.
19. Air dry thoroughly.
20. Mount slides in an organic mounting media such as Permount.

Electron Microscopy

See references for suggested electron microscopy protocols.²

REFERENCES

1. Geoghegan and Ackerman, *J Histo/Cytochemistry*, 25(11):1187-1200, 1977.
2. Oliver, C. (1994) in: Javois, L.C. ed. Methods in Molecular Biology, Vol. 34: Immunocytochemical Methods and Protocols, Humana Press, Totowa, NJ.

PRODUCT SAFETY AND HANDLING

This product is considered non-hazardous as defined by the Hazard Communication Standard (29 CFR 1910.1200). Avoid contact with skin and eyes. In case of contact or spillage, clean with copious amounts of water. Product may be disposed via sanitary sewer.

TROUBLESHOOTING

A crystalline precipitate can form upon long term storage at 2 - 8°C. The precipitate can be removed by centrifugation or by filtering. This will not affect performance.

PROBLEM	POSSIBLE CAUSE	CORRECTIVE ACTION
Background	Excess antibody; reaction too fast.	-Dilute primary antibody and/or gold conjugate. -Reduce incubation times.
Floating Precipitate	-Excess antibody; reaction too fast. -Silver enhancer incubation too long.	-Dilute primary and/or gold conjugate -Reduce incubation times
Weak Staining	-Insufficient binding. -Excessive washing.	-Increase incubation times. -Reduce washing times.
Purple or Other Color	-Excess Counterstain.	-Shorten counterstain incubation time.

PROBLEM	POSSIBLE CAUSE	CORRECTIVE ACTION
Fading	-Excess antibody; reaction too fast. -Inappropriate wash buffer. -Inappropriate mounting media. -Storage Condition.	- Dilute primary antibody and/or gold conjugate. -Use Tris-HCl or PBS. -Use only an organic mounting media. -Store in the dark.

RELATED PRODUCTS

Gold Labeled Goat anti-Mouse System, 5 nm	Cat. No. 58-18-06
Gold Labeled Goat anti-Rabbit System, 5 nm	Cat. No. 58-15-06
Gold Labeled Goat anti-Biotin System, 5 nm	Cat. No. 58-40-06
Gold Labeled Goat anti-Human System, 5 nm	Cat. No. 58-10-06
Silver Enhancer Kit for Microscopy	Cat. No. 50-22-01
Contrast Red	Cat. No. 71-00-05
Contrast Green	Cat. No. 71-00-11
Contrast Blue	Cat. No. 71-00-06
Goat Serum Block	Cat. No. 71-00-27

See KPL's catalog for a wide selection of antibodies and immunohistochemical substrates.

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