Horseradish Peroxidase Labeled
Streptavidin

molecular biology grade

Catalog No.	Size
474-3000	1 mL

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 has been shown to bind four molecules of biotin with high affinity ($K_p=10^{-15}$ M^{-1}). Electrophoretically pure streptavidin is linked to horseradish peroxidase using a modification of the periodate method of Nakane and Kawaoi (1).

FORM

The conjugate is provided in a liquid buffer containing a proprietary stabilizer and an anti-bacterial agent. It is prepared with molecular biology grade chemicals and dispensed into RNase/DNase-free sterile vials.

STORAGE/STABILITY

Store at 2-8°C. Stable for a minimum of 1 year from date of receipt at 2-8°C as an undiluted liquid. Dilute only immediately before use.

ENZYME:PROTEIN RATIO

Molar peroxidase:streptavidin ratio ≡ 2.5:1.

CONCENTRATION

The concentration of streptavidin is 0.1 mg/ml in a 1.0 ml volume.

APPLICATIONS

Peroxidase labeled streptavidin is suitable for use in Northern blotting, Southern blotting, plaque and colony hybridizations, *in situ* hybridization, and immunohistochemistry (brief protocols described below). This conjugate may also be used for ELISA and immunoblotting procedures (See References 2-5).

SUGGESTED WORKING DILUTIONS

Different assay conditions require that serial dilutions of all reagents be performed to determine optimal working concentrations. Prepare the working dilution immediately before use. Storage at a working dilution may result in enzyme inactivation and performance loss. Do not use sodium azide in the diluent. For suggested starting dilutions, see the appropriate protocol.

SUGGESTED PROTOCOLS

All steps are at room temperature unless otherwise noted.

Southern Blotting, Northern Blotting, Plaque and Colony Hybridizations:

Following hybridization with a biotinylated probe and post-hybridization washing:

1. Place membrane in a small container or hybridization bag, block with KPL's Detector Block (See RELATED PRODUCTS), or other appropriate blocking solution, for 30 minutes.
2. Dilute peroxidase labeled streptavidin 1:100-1:1000 in blocking solution. Incubate membrane for 20 minutes with diluted HRP-SA, use at least 0.25 ml per cm^2 membrane. The optimal dilution of HRP-SA must be determined experimentally.
3. Transfer membrane to a clean container and wash with KPL's Biotin Wash Solution (See RELATED PRODUCTS). Wash 3 times for 5 minutes each using at least 0.4 ml wash solution per cm^2 membrane.
4. Detect using TMB Membrane Substrate, LumiGLO® Chemiluminescent Substrate (See RELATED PRODUCTS) or other appropriate peroxidase substrate following appropriate protocols.

***In Situ* Hybridization:**

Following hybridization of tissue or cells with a biotinylated probe and post-hybridization washing:

1. Dilute peroxidase labeled streptavidin 1:20-1:200 in an appropriate diluent and apply approximately 100 µl to specimen. Cover slide to prevent evaporation. Incubate in a 37°C humidified chamber for 20 minutes. The optimal dilution of HRP-SA must be determined experimentally.
2. Immerse slide in a coplin jar containing KPL's Biotin Wash Solution (See RELATED PRODUCTS), or other appropriate wash solution. Wash 3 times for 5 minutes each.
3. Detect using TrueBlue® (See RELATED PRODUCTS) or other appropriate peroxidase substrate following manufacturer's instructions.
4. Apply a red counterstain, such as Orcein or Eosin (See RELATED PRODUCTS), according to manufacturer's instructions.

5. Dehydrate and mount the slide with an organic mounting media such as Permount.

Immunohistochemistry:
Following incubation of the specimen with primary and biotin-labeled secondary antibody:

1. Dilute peroxidase labeled streptavidin 1:10-1:100 in Goat Serum Block (See RELATED PRODUCTS) or 10% normal goat serum in 0.01 M Tris, pH 7.65. Flood the slide with diluted peroxidase labeled streptavidin. Incubate at room temperature for 30 minutes. The optimal dilution of HRP-SA must be determined experimentally.
2. Soak the slide in PBS or Tris-HCl for 5 minutes.
3. Detect using DAB, *Stable*DAB®, TrueBlue®, HistoMark® ORANGE, HistoMark®BLACK (See RELATED PRODUCTS) or other appropriate peroxidase substrate.
4. Counterstain if desired.

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 a sanitary sewer.

REFERENCES

1. Nakane, P.K. and Kawaoi, A. (1974). *J. Histochem. Cytochem*. 22: 1084-1091.
2. Brigati, D.J., et. al. (1983) *Virology*, 126: 32-50.
3. Harlow, E. and Lane, D. (1988) Antibodies: A Laboratory Manual. Cold Spring Harbor Laboratory Press, NY.
4. Sambrook, J. et. al. (1989) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, NY.
5. Crowther, J.R. (1995) Methods in Molecular Biology: ELISA: Theory and Practice. Vol. 42, Humana Press, NJ.

RELATED PRODUCTS

Detector Block	Cat. No. 71-83-00
Biotin Wash Solution (10X)	Cat. No. 50-63-06
Goat Serum Block	Cat. No. 71-00-27
LumiGLO® Chemiluminescent Substrate	Cat. No. 54-61-02
TMB Membrane Substrate	Cat. No. 50-77-03

Counterstains:

Orcein	Cat. No. 71-01-01
Eosin	Cat. No. 71-02-01

Immunohistochemistry Substrates:

DAB	Cat. No. 54-10-00
*Stable*DAB®	Cat. No. 54-11-00
TrueBlue®	Cat. No. 50-78-02
HistoMark® ORANGE	Cat. No. 54-74-00
HistoMark® BLACK	Cat. No. 54-75-00

See KPL's catalog for a complete list of biotinylated antibodies, substrates, and complete kits for immunohistochemistry, Southern blotting, Northern blotting and *in situ* hybridization.

L-251-05

910 Clopper Road, Gaithersburg, MD, USA 20878
301-948-7755 • 800-638-3167 • Fax 301-948-0169 • www.kpl.com