Qty: 2.5 mg/2 ml

ZyMax[™] Streptavidin-HRP Conjugate

Catalog No. 43-8323 Lot No.

ZYMED® Laboratories

invitrogen immunodetection

HRP-Streptavidin Conjugate (ZyMax[™] Grade)

FORM

ZyMax[™] Streptavidin-HRP is supplied as a liquid 2 ml aliquot (1.25 mg/ml) in a proprietary enzyme conjugate stabilizing buffer containing 0.1% proclin as a preservative. In the preparation of this product, Zymed uses highly purified Streptavidin and ultrapure horseradish peroxidase (RZ > 3.0).

BACKGROUND

Streptavidin (MW \equiv 66 kDa) binds specifically with biotin (244 Da). It is derived from the bacterium *Streptomyces avidinii* and bears a remarkable similarity to chicken egg-white avidin both in three-dimensional structure and its ability to bind biotin with extremely high affinity ($K_d=10^{-15}M$). It is a tetrameric protein capable of binding up to 4 biotin molecules. Unlike avidin, Streptavidin is non-glycosylated and is essentially neutral in charge, whereas avidin ($pl \cong 10.5$) is basic at neutral pH. Because of this, streptavidin has considerably less non-specific binding resulting in less background. It has replaced avidin as the reagent of choice for most applications where protein interactions may cause background. Under conditions where background is extremely problematic, investigators should consider using Z-Avidin, a modified form of avidin that may exhibit even greater sensitivity in tissue staining and flow cytometry--see Related Products, below.

USAGE

ZyMax[™] grade reagents offer enhanced assay sensitivity, higher titers, and greater flexibility in assay design, than standard grade reagents. ZyMax[™] Streptavidin-HRP is recommended for use in detection systems utilizing biotinylated antibodies and other biotinylated molecules. This product is documented for use in a variety of common applications including immunohistochemistry, Western blotting, *In Situ* hybridization, and ELISA. For an excellent, comprehensive review of properties and applications of streptavidin/biotin amplification methods, see Bayer and Wilchek's <u>Avidin-Biotin Technology</u>, Methods in Enzymology, Volume 184, Academic Press (1990).

Working concentrations for specific applications should be determined by the investigator. Appropriate dilutions will be affected by several factors, including primary and secondary antibody affinity, antigen concentration and length of incubations. We recommend the following ranges as starting points.

| Immunohistochemistry⁽¹⁻⁶⁾: 1:150 to 1:750 | Western blot (chromogenic)⁽¹²⁾: 1:1,250 to 1:7,500 | Western blot (chemiluminescence)⁽⁸⁻¹¹⁾: 1:2,500 to 1:15,000 | ELISA⁽¹³⁻¹⁶⁾: 1:2,000 to 1:15,000 |

PROCEDURES

Zymed has general guidelines for ELISA, blotting and other applications available on our Web site at www.invitrogen.com/methods. You may also obtain assistance from our Technical Service department at (800) 955-6288. Another good source of information about general immunoassay procedures is Ed Harlow & David Lane's Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory (1988). Also see Bayer and Wilchek (referenced above).

(43-8323 continued)

STORAGE

The undiluted product is stable when stored at 2-8°C. The stabilizing buffer contains glycerol, allowing storage of the product at -20°C without freezing. Zymed does not guarantee performance of this product beyond the expiration date on the bottle.

WARRANTY

Zymed products are guaranteed to perform as stated for the recommended applications. This warranty is valid up until the expiration date printed on the bottle.

SELECTED ZYMED PRODUCT REFERENCES

References cite Zymed's standard grade product--ZyMax™ streptavidin conjugates can be used at a higher dilution.

Immunohistochemistry & Immunocytochemistry

- 1. McCullough, K.D. et al, Cancer Research 57:1807-1813 (1997).
- 2. Simsir, A. et al, J Histotech. 19(4):335-337 (1997).
- 3. Tullin, S. et al, *J Immunol*. 158:5554-5559 (1997).
- 4. Ibanes, J.D. at al., Vet. Pathol. 33:412-418 (1996).
- 5. Muss, H.B. et al, New Eng. J Medicine 330:1260-1266 (1994).
- 6. Xu, M et al, Cell 79:729-742 (1994).

In Situ Hybridizaion

7. McQuid, S. and Allan, G., J. Histochem. Cytochem. 40:569-574 (1992)

Western blot (with chemiluminescence)

- 8. Murakami, M. et al, J Immunol. 159:439-446 (1997).
- 9. Solheim, J.C. et al, J Immunol. 158:2236-2241 (1997).
- 10. Tamura, A. et al, J Immunol. 155:499-507 (1995).
- 11. Gettner, S. et al, J Cell Biol. 129(4):1127-1141 (1995).

Western blot (chromogen only)

12. Scarpa et al, Exp. Cell Res. 229:147-154 (1996).

ELISA

- 13. Dittel, B. et al, J Immunol. 158:4065-4073 (1997).
- 14. Koo, G.C. et al, J Immunol. 158:5120-5128 (1997).
- 15. Hicks, D.G. et al, J Histotech. 20(3):215 (1997).
- 16. Lindner, M. et al, J Neurosci. 14(4):2282-2289 (1994).

RELATED PRODUCTS

Product	Cat. No.
Z-Avidin-HRP	43-4523
Rabbit anti-Mouse IgG-Biotin (ZyMax™ Grade)	81-6540
Goat anti Rabbit IgG-Biotin (ZyMax Grade)	81-6140
Rabbit anti-Goat IgG-Biotin (ZyMax Grade)	81-1640
Goat anti-Human IgG-Biotin (ZyMax Grade)	81-7140
Goat anti-Rat IgG-Biotin	62-9540
Protein A-Biotin	10-1040
rec-Protein G-Biotin	10-1240
ABTS Single Solution Chromogen	00-2024 (ELISA)
TMB Single Solution Chromogen	00-2023 (ELISA), 00-2019 (blot)
DAB Liquid Reagent Chromogen Set	00-2014 (IHC)
AEC Single Solution Chromogen	00-1111 (IHC), 00-2022 (blot)
4-CN Single Solution Chromogen	00-2025 (blot)

TRADEMARKS

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For Research Use Only

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