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ProductInformation

Streptavidin-Peroxidase Polymer, Ultrasensitive

Product Number **S 2438**
Store at –20 °C

TECHNICAL BULLETIN

Product Description
This product is prepared by covalently conjugating streptavidin and horseradish peroxidase (HRP) to a hydrophilic polymer backbone. Multiple active biomolecules on each polymer chain increase the biotin binding capacity and amplify the peroxidase enzyme signal compared to conventional streptavidin-HRP formulations. This conjugate is useful for highly sensitive detection of biotinylated proteins, DNA, RNA, and other biomolecules.

This polymer conjugate can be used in applications where streptavidin-HRP conjugates are currently used, such as Western blot, dot blot, and ELISA assay formats. The conjugate can also be used in immunohistochemistry and immunocytochemistry for detection of surface expressed antigens.

This product is supplied as a 1.0 mg protein/ml solution containing 0.01 M sodium phosphate, pH 7.4, 0.15 M sodium chloride, 50% (v/v) glycerol, stabilizer, and preservative.

Precautions and Disclaimer
For research use only, not for use in diagnostic procedures.

Storage/Stability
The product should be stored as supplied at –20 °C. Upon dilution in an appropriate buffer, the conjugate should be used immediately.

Preparation Instructions
Dilute the streptavidin-HRP polymer conjugate to the recommended working dilution in Phosphate Buffered Saline (PBS), pH 7.4 (P 3813) or PBS with 0.05% TWEEN 20 (P 3563).

Suggested working dilution: A dilution of 1:200 – 1:1000 may be used for most applications. It is recommended that each user determine the optimal conjugate concentration in their system to maximize detection sensitivity and to minimize background.

Procedure
Sample protocol for Western Blot

1. Perform SDS-PAGE and membrane transfer of biotinylated proteins using standard conditions. PVDF, Immobilon-P, or nitrocellulose membranes may be used as appropriate.

2. Block the membrane with 1% BSA (A 9647) in PBS with 0.05% TWEEN 20 at room temperature for 1 hour.

3. Wash the membrane two times for 5 minutes each using PBS with 0.05% TWEEN 20.

4. Incubate the membrane with streptavidin-HRP polymer conjugate diluted 1:1000 in PBS with 0.05% TWEEN 20 at room temperature for 1 hour.

5. Wash the membrane six times for 5 minutes each using PBS with 0.05% TWEEN 20.

6. Incubate the membrane with an appropriate chemiluminescent or precipitating colorimetric substrate using standard conditions. Detect according to manufacturer recommendations.

Sample Protocol for ELISA (96 well format)

1. Prepare a solution of the biotinylated protein in 0.05 M $NaHCO_3$, pH 9.6 (C 3041). A protein concentration of 1 –10 µg/ml should be used if the optimal concentration is not known.

2. Coat a multiwell plate with 100 µl to 200 µl per well of the sample solution and incubate 2 to 4 hours at room temperature or overnight at 4 °C.

3. Wash the wells three times, 300 µl per well, using PBS with 0.05% TWEEN 20.

4. Incubate the wells with an appropriate blocking solution such as PBS with 1% BSA (P 3688) at room temperature for one hour. Use a volume, which is slightly more than that used to coat the wells in step 2.
5. Wash the wells three times, 300 µl per well, using PBS with 0.05% TWEEN 20.

6. Prepare a working solution of the streptavidin-HRP polymer conjugate by diluting 1:200 in PBS with 0.05% TWEEN 20. To assess background from non-specific binding of conjugate, prepare a similar dilution of the streptavidin-HRP polymer conjugate in PBS with 0.05% TWEEN 20 containing 0.05 µg/ml biotin (B 4501).

7. Add 200 µl per well of the streptavidin-HRP polymer conjugate solutions to the plate. Include wells containing only PBS with 0.05% TWEEN 20 (no conjugate) to determine subsequent substrate background. Incubate at room temperature for one hour.

8. Wash the wells five times, 300 µl per well, using PBS with 0.05% TWEEN 20.

9. Incubate the wells with 200 µl per well of an appropriate soluble colorimetric or chemiluminescent substrate. Detect according to manufacturer recommendations.

Common Peroxidase substrates

	Western Blot	**ELISA**
Colorimetric Substrates	TMB (T 0565) DAB (D 7304) AEC (AEC-101)	TMB (T 8665) ABTS (A 3219) OPD (P 8287)
Chemiluminescent Substrates and Activators	Luminol/ (A 8511) Iodophenol/peroxide	
Fluorescent Substrates	4-HPPA/ (H 6386) peroxide	

References

1. Graf, R. and Friedl, P., Detection of immobilized proteins on nitrocellulose membranes using a biotinylation-dependent system. Anal. Biochem., **273**, 291-297 (1999)
2. Walsh, P.S., et al., A rapid chemiluminescent method for quantitation of human DNA. Nucleic Acids Res., **20**, 5061-5065 (1992).
3. Kellner, U., et al., Detection of four new single-stranded telomeric DNA binding proteins by means of an optimized protein blotting procedure. Pathol. Res. Pract., **196**, 801-806 (2000).

Troubleshooting Guide

Problem	Possible Cause	Solution
High background	Non-specific binding of protein in target protein solution	Decrease concentration of target protein solution. Add blocking agents (e.g. BSA, casein, gelatin, or ovalbumin) at 0.05 to 1% in the diluent.
	Inappropriate wash protocol	Increase the number of washes. Use more stringent washes, e.g. include detergents such as 0.1% TWEEN 20 or 0.1% Triton X-100 in the wash buffer.
	Non-specific binding of conjugate	Decrease conjugate concentration. Add blocking agents to diluent.
	Substrate contamination/ degradation	Decrease the substrate incubation time. Use fresh substrate solution.
Low signal	Insufficient target protein captured	Increase concentration of target protein solution. Increase incubation time with target protein.
	Conjugate concentration too low	Increase conjugate concentration

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