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Datasheet for S000-03**Streptavidin Peroxidase Conjugated****Overview**

Description:	Streptavidin Peroxidase Conjugated - S000-03
Item No.:	S000-03
Size:	1 mg
Applications:	Dot Blot, WB, ELISA, EM, IF, IHC, Other

Product Details

Background:	Streptavidin is isolated from bacteria, <i>Streptomyces avidinii</i> , and has an exceptionally high binding affinity for B7 (biotin). Rockland offers streptavidin in unconjugated and conjugated forms for common immunoassays including ELISA, western blotting, immunohistochemistry. Streptavidin is a tetrameric protein capable of binding 4 biotin groups to each molecule of streptavidin. While streptavidin has identical binding properties as avidin, it lacks the glycoprotein portion of the molecule and therefore shows less non-specific binding. Streptavidin is a slightly smaller molecule with a molecular weight of approximately 53.6 kDa. The sequence of avidin only shows 30% homology with streptavidin, and anti-avidin and anti-streptavidin antibodies are not immunologically cross reactive. Horseradish Peroxidase (HRP) is an enzyme that utilize organic peroxide compounds as electron donors. Naturally provides protection for plants against pathogens, but can be utilized in molecular biology to convert various substrates to detectable compounds (such as in Western Blotting and ELISAs).
Synonyms:	HRP-SA, Horseradish Peroxidase conjugated S avidin, Streptavidin HRP, Streptavidin conjugated to horseradish peroxidase (HRP), HRP-linked Streptavidin
Conjugate:	Peroxidase (HRP)

Target Details

Purity/Specificity:	Streptavidin-HRP was prepared from chromatographically purified streptavidin. Streptavidin Peroxidase conjugate was assayed by immunoelectrophoresis resulted in a single precipitin arc against anti-Peroxidase and anti-Streptavidin.
Relevant Links:	• S000-03 SDS • UniProtKB - P22629 • NCBI - CAA00084.1

Application Details

Tested Applications:	Dot Blot, WB
Suggested Applications:	ELISA, EM, IF, IHC, Other (Based on references)
Application Note:	Streptavidin Peroxidase has been tested by dot blot and western blot and is a useful detection reagent for primary antibodies conjugated to biotin. Streptavidin Peroxidase can be utilized in Immunohistochemistry, Immunofluorescence, immuno-EM, Western Blotting, and ELISA experiment formats in combination with the proper substrate (TMB-1000 or FEMTOMAX-110).
Assay Dilutions:	All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.
ELISA:	1:20,000 - 1:200,000
IF:	User Optimized
IHC:	1:1,000 - 1:5,000
WB:	1:10,000 - 1:40,000

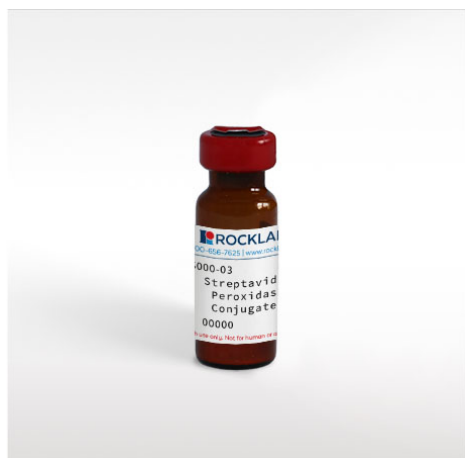
Formulation

Physical State:	Lyophilized
Concentration:	1.0 mg/mL by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Gentamicin Sulfate. Do NOT add Sodium Azide!
Stabilizer:	10 mg/mL Bovine Serum Albumin (BSA) - Immunoglobulin and Protease free
Reconstitution Volume:	1.0 mL
Reconstitution Buffer:	Restore with deionized water (or equivalent)

Shipping & Handling

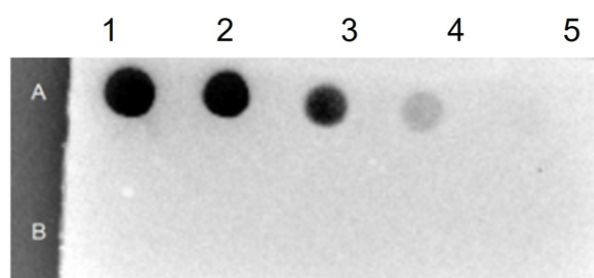
Shipping Condition:	Ambient
Storage Condition:	Store vial at 4° C prior to restoration. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. Streptavidin Peroxidase conjugated is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

Images



Bottle

Streptavidin Peroxidase Conjugated



Dot Blot

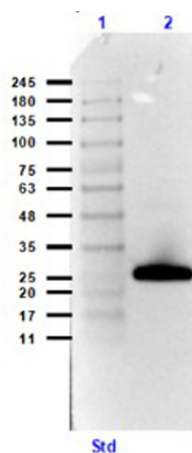
Dot Blot Results of Streptavidin Peroxidase Conjugate.

Row A: BSA-Biotin Conjugated.

Row B: BSA.

Sample dilutions: 1- 100ng, 2- 33.33ng, 3- 11.11ng, 4-3.7ng, 5- 1.23ng.

Streptavidin Peroxidase Conjugated at 1.0µg/mL for 30mins at RT.



Western Blot

Western Blot Results using Streptavidin Peroxidase Conjugate and Goat Anti-GST Biotin Conjugate Antibodies.

Lane 1: Opal prestained molecular weight ladder (p/n MB-210-0500).

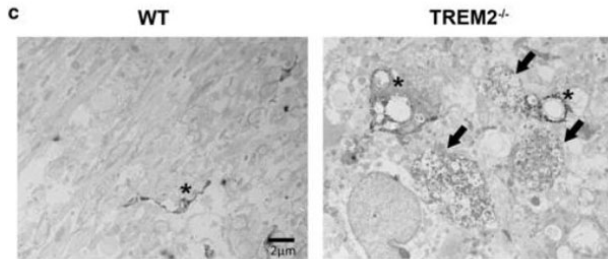
Lane 2: GST (p/n 000-001-200) [0.05 µg].

Primary Antibody: Goat Anti-GST Biotin Conjugate (p/n 600-106-200) at 1.0µg/mL overnight at 4°C.

Secondary Antibody; Streptavidin Peroxidase Conjugate at 1:40,000 for 30mins at RT.

Block: Blocking Buffer for Fluorescent Western Blotting (p/n MB-070) for 30mins at RT.

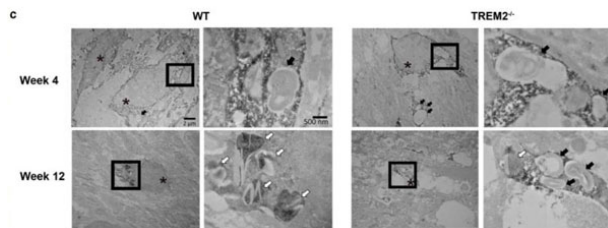
Exp: 5 sec.



Immunocytochemistry

Immuno-electron microscopy using biotinylated anti-rabbit and streptavidin-HRP.

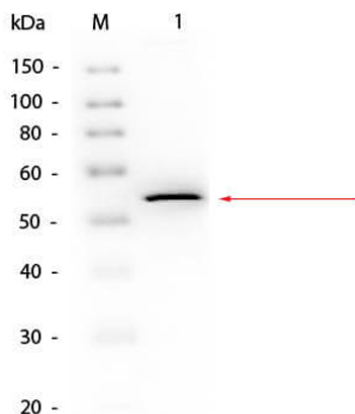
TREM2^{-/-} mice show more severe axonal pathology after CPZ. (c) EM images of WT and TREM2^{-/-} at 12 weeks of CPZ treatment. Black arrows indicate dystrophic autophagocytic axons and asterisks indicate Iba1+ immunolabeled microglia. Fig. 2. PMID: 25631124.



Immunocytochemistry

Immuno-electron microscopy using biotinylated anti-rabbit and streptavidin-HRP.

Defect in myelin degradation in TREM2^{-/-} microglia. (c) Immuno-EM images of TREM2^{-/-} and WT microglia stained with Iba1 in the CC at 4 and 12 weeks on CPZ treatment. Images on the left in WT and TREM2^{-/-} panels at week 4 and 12 (3,000× magnification) depict Iba + microglial cells (asterisks). A higher magnification (15,000×) for the boxed area is shown on the right of each image. Black arrows indicate phagosomes containing myelin debris. White arrows indicate pi granules. Fig. 7. PMID: 25631124.

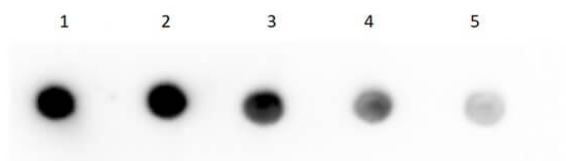


Western Blot

Western Blot of Goat anti-Glycerol Kinase Antibody Biotin Conjugated using Streptavidin HRP. Lane 1: Glycerol Kinase. Load: 50 ng per lane. Primary antibody: Glycerol Kinase Antibody Biotin Conjugated at 1:1000 overnight at 4°C. Secondary antibody: HRP Streptavidin (p/n S000-03) secondary antibody at 1:40,000 for 30 min at RT. Block: MB-070 for 30 min at RT. Predicted/Observed size: 55 kDa, 55 kDa for Glycerol Kinase.

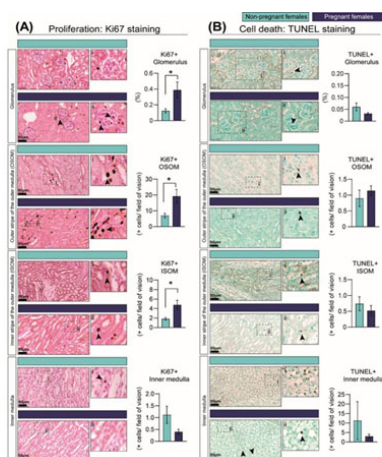
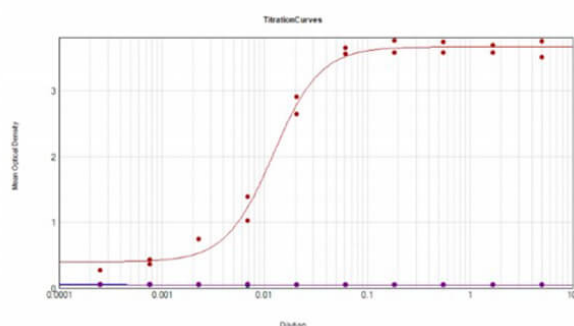
Dot Blot

Dot Blot of Human IgG F(c) Fragment Biotin Conjugated using Streptavidin HRP. Human IgG F(c) Biotin Conjugate (1) 100ng, (2) 33.33ng, (3) 11.11ng, (4) 3.70ng, (5) 1.23ng. Primary Antibody: none. Secondary Antibody: Streptavidin HRP (p/n S000-03) at 1:40,000 for 30 mins at RT. Block: BlockOut buffer (p/n MB-073) at RT for 30 mins. Exposure: 1 sec.



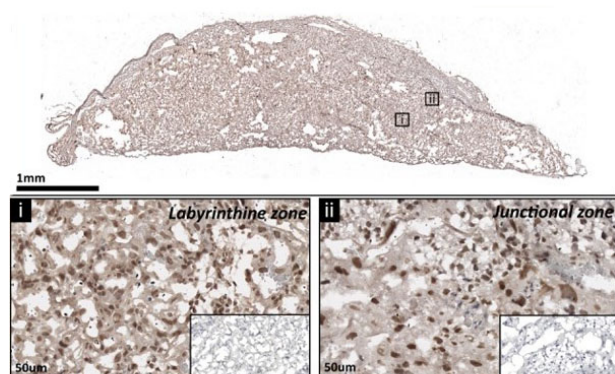
ELISA

ELISA Results of Human IgG Whole Molecule Biotin Conjugated using Streptavidin-HRP. Each well was coated in duplicate with 1.0 µg of Human IgG Whole Molecule Biotin Conjugate. The working dilution is 82,800. The starting dilution of antibody was 5µg/ml and the X-axis represents the Log10 of a 3-fold dilution. This titration is a 4-parameter curve fit where the IC50 is defined as the titer of the antibody. Assay performed using Streptavidin-HRP (p/n S000-03) and TMB substrate (p/n TMBE-1000).



Immunohistochemistry

Percentage of cells showing cell-cycle activation as informed by Ki67 immunostaining (A) and cell death as identified by TUNEL assay (B) in the kidney in response to pregnancy in mice. Representative image of stained kidney from non-pregnant and pregnant female mice. Data are presented as mean ± SEM (n = 5/group). Asterisks represent significant differences between non-pregnant and pregnant mice as determined by Student's t-Test (* p < 0.05). Slides incubated with goat anti-rabbit secondary antibody (1:1000) and streptavidin-horseradish peroxidase (1:500, p/n S000-03). Images with the labels i and ii depict high magnification of the selected area. Arrow heads indicate positive DAB staining. Scale bar is 50 µm. Figure 3. PMID: 35682969.



Immunohistochemistry

The expression of p110 α protein by the mouse placenta on day 19 of pregnancy. Representative stained section shown with negative control shown in the figure inset. For localization of p110 α , placental sections were washed with PBS to remove OCT and underwent antigen retrieval with citrate buffer before immunolabelling against p110 α . Sections were treated with 0.5% Triton X-100 before immunolabelling. Bound antibody was detected using biotinylated goat anti-rabbit IgG followed by streptavidin-conjugated horseradish peroxidase (p/n S000-03) and 3,3'-diaminobenzidine (DAB). Sections were lightly counterstained with hematoxylin and mounted in DPX. Supplement Fig 1. PMID: 31241463.



Western Blot

Western Blot of Peroxidase Conjugated Streptavidin. Lane 1: Human IL-7. Load: 50 ng per lane. Primary antibody: Human IL-7 Biotin Conjugated antibody at 1:1,000 for overnight at 4°C. Secondary antibody: Peroxidase Conjugated Streptavidin at 1:40,000 for 30 min at RT. Block: 5% BLOTTO 30 min at RT. Predicted/Observed size: 17 kDa, 17 kDa for Human IL-7. Other band(s): none.

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