



Applications Species Cross-Reactivity W All

Description: This Cell Signaling Technology product is useful for the detection of biotinylated proteins (1,2). Conjugation of horseradish peroxidase (HRP) to streptavidin is obtained by cross linking the amino groups on streptavidin with the carbohydrate groups on HRP.

Background: Streptavidin is a 53 kDa homotetramer isolated from *Streptomyces avidinii* for use in isolation and detection bioassays (3). Each streptavidin subunit forms high affinity non-covalent bonds with the vitamin biotin. Because of its strong non-covalent interaction with biotin, streptavidin can be used to detect and isolate biotinylated proteins (1,2).

Chemiluminescent detection systems have emerged as the best all-around detection method for use with western blots and ELISA. These detection assays eliminate the hazards associated with radioactive materials and toxic chromogenic substrates. The speed and sensitivity of these methods are unequalled by traditional alternatives. Streptavidin-HRP is used with biotinylated proteins and specific chemiluminescent substrates to generate light signal. Streptavidin-HRP conjugates have a very high turnover rate, coupling high sensitivity with short reaction times

Specificity/Sensitivity: Streptavidin has a remarkably high affinity for its natural ligand, biotin. The complex and irregular structure of the biotin-binding site makes it highly optimized for biotin binding and confers great specificity to the streptavidin-biotin complexes (4).

Source/Purification: Streptavidin-HRP is purified by gel filtration. The conjugate product is free from unconjugated streptavidin and HRP.

Background References:

room temperature shaking.

- Updyke, T.V. and Nicolson, G.L. (1984) J Immunol Methods 73, 83–95.
- (2) Buckie, J.W. and Cook, G.M. (1986) *Anal Biochem* 156, 463–72.
- (3) Chaiet, L. and Wolf, F.J. (1964) Arch Biochem Biophys 106, 1–5.
- (4) Reznik, G.O. et al. (1998) Proc Natl Acad Sci USA 95, 13525–30.



Western blot analysis of extracts from HeLa cells, untreated or treated with Paclitaxel #9807, using Phospho-Histone H3 (Ser10) (D2C8) Rabbit mAb (Biotinylated) #3642 and Streptavidin-HRP #3999 for detection.

Swiss-Prot Acc. #P22629

Western blotting

Storage: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 2 mg/ml bovine serum albumin (BSA) and 50% glycerol. Store at -20° C.

Recommended Antibody Dilutions:

1:2000

For application specific protocols please see the web page for this product at www.cellsignal.com.

Please visit www.cellsignal.com for a complete listing of recommended companion products.

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 Applications Key:
 W—Western
 IP—Immunoprecipitation
 IHC—Immunohistochemistry
 ChIP—Chromatin Immunoprecipitation
 IF—Immunofluorescence
 F—Flow cytometry
 E-P—ELISA-Peptide

 Species Cross-Reactivity Key:
 H—human
 M—mouse
 R—rat
 Hm—hamster
 Mk—monkey
 Mi—mink
 C—chicken
 Dm—D. melanogaster
 X—zebrafish
 B—bovine

 Dg—dog
 Pg—pig
 Sc—S. cerevisiae
 Cene-C. elegans
 Hr—Horse
 AII—all species expected

IMPORTANT: Dilute Streptavidin-HRP in 5% nonfat dry milk, 1X TBS, 0.1% Tween 20. Incubate for 1 hour at