Store at -20°C

#7727

Biotinylated Protein Ladder Detection Pack

Includes Biotinylated Protein Ladder in Premixed Format and Anti-biotin, HRP-linked Antibody

☐ Small 650 µl

☐ Large 5 x 650 µl

Cell Signaling TECHNOLOGY®

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For Research Use Only. Not For Use In Diagnostic Procedures.

Product Includes	Product #	Qty (Small)	Qty (Large)
Biotinylated Protein Ladder	81851	650 ul	5 x 650 ul
Anti-biotin, HRP-linked Antibody	7075	650 ul	5 x 650 ul

Description: Biotinylated Protein Ladder Detection Packs are designed to detect the molecular weight ladders on Western blots when using the horseradish peroxidase (HRP) based Western detection system. The pack has been optimized for chemiluminescent Western detection procedures.

The molecular weight ladders are a mixture of purified proteins covalently coupled to biotin that resolve to 10 bands that have a size range of 9-200 kDa. The anti-biotin antibody is used to detect biotinylated protein ladders on Western blots.

Specificity/Sensitivity: The Anti-biotin, HRP-linked Antibody may cross react with endogenously expressed biotinylated proteins. Careful titration may be needed.

Source/Purification: The protein ladder consists of 10 proteins ranging in apparent molecular weight from 9 to 200 kDa. The 9 kDa protein is corresponding to aprotinin purified from bovine lung. The proteins from 20-50 kDa are paramyosin fragments; the higher molecular weight proteins are fusions of maltose binding protein (MBP) with paramyosin or paramyosin/lacZ fragments. The 200 kDa protein is corresponding to alpha-2-macroglobulin purified from human plasma.

The anti-biotin HRP-linked antibody should only be used to detect the marker and not other biotinylated proteins.

Solutions and Reagents:

Biotinylated Protein Ladder: Supplied in 62.5 mM Tris-HCl (pH 6.8 at 25°C), 2% SDS (w/v), 40 mM dithiothreitol (DTT), 0.01% (w/v) phenol red and 10% glycerol. Store at –20°C.

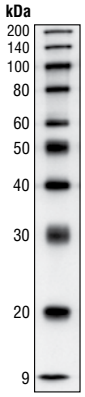
Anti-biotin, HRP-linked Antibody: Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 2 mg/ml bovine serum albumin (BSA) and 50% glycerol. Store at –20°C.

Directions for Use:

(a) Thaw protein ladder on ice. Mix well and aliquot the desired amount of the protein ladder (10 µl for mini-gels and 20 µl for full length gels) into a separate tube.

(b) Heat the ladder to 95-100°C for 2 minutes.

(c) After a quick microcentrifuge spin, load directly onto gel. To ensure uniform mobility, load an equal volume of 1X Reducing SDS Loading Buffer into any unused wells.



11% SDS-PAGE

Detection of Protein Ladders:

(a) Perform chemiluminescent detection according to the manufacturer's instructions except, at the stage of secondary antibody incubation with the blot, concurrently add the anti-biotin, HRPlinked antibody (provided) at a dilution of 1:1000. Incubate for one hour at room temperature with gentle agitation.

(b) Proceed as usual with the detection procedure.

Storage: See the "Solutions and Reagents."

Recommended Antibody Dilution:

1:1000

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

Background References:

(1) Sambrook, J., Fritsch, E.F. and Maniatis, T. Molecular Cloning: A Laboratory Manual, second edition, Cold Spring Harbor Laboratory.

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**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—horse **All**—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.