

Biotinylated Protein Ladder

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

- Small 65 mini-blots
 Large 325 mini-blots

Orders ■ 877-616-CELL (2355)
orders@cellsignal.com
Support ■ 877-678-TECH (8324)
info@cellsignal.com
Web ■ www.cellsignal.com

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This product is for *in vitro* research use only and is not intended for use in humans or animals.

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 contains biotinylated protein ladders and HRP-linked, anti-biotin antibody. 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. The packs have been optimized for chemiluminescent Western detection procedures.

Source/Purification: The protein ladder consists of 10 engineered proteins ranging in apparent molecular weight from 9 to 200 kDa. The proteins from 9-50 kDa are paramyosin fragments; the higher molecular weight proteins are fusions of maltose binding protein (MBP) with paramyosin or paramyosin/lacZ fragments.

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

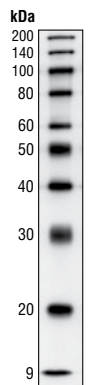
Solutions and Reagents:

Biotinylated Protein Ladder: Supplied in 65 mM Tris-HCl (pH 7.0 at 25°C), 35 mM NaCl, 1 mM Na₂EDTA, 2% SDS (w/v), 1 mM NaN₃, 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 µM sodium HEPES (pH 7.5 at 25°C), 150 mM NaCl, 100 µg/ml bovine serum albumin (BSA) and 50% glycerol. Store at -20°C.

Directions for Use:

- 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.
- Heat the ladder to 95-100°C for 2 minutes.
- 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:

- 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, HRP-linked antibody (provided) at a dilution of 1:1000. Incubate for one hour at room temperature with gentle agitation.
- Proceed as usual with the detection procedure.

Background References:

- Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, second edition, Cold Spring Harbor Laboratory.

Storage: See the "Solutions and Reagents."

Recommended Antibody Dilution:

1:1000

Companion Products:

Anti-biotin, HRP-linked Antibody #7075

Phototope®-HRP Western Blot Detection System, Anti-rabbit IgG, HRP-linked Antibody #7071

Phototope®-HRP Western Blot Detection System, Anti-mouse IgG, HRP-linked Antibody #7072