

## ProSieve Protein Marker: Western Blot Detection

### Instructions for Conjugating AP and HRP to S-Tag

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ProSieve Protein Markers consist of a novel set of proteins designed for accurate sizing of protein samples in SDS-PAGE. The markers contain 10 proteins with exact masses of 5 kDa, 10 kDa, 15 kDa, 25 kDa, 35 kDa, 50 kDa, 75 kDa, 100 kDa, 150 kDa, and 225 kDa. The 50 kDa band is double-intensity for easy identification.

ProSieve Protein Marker recombinant proteins have an S-Tag (K E T A A A K F E R Q H M D S) attached which can be detected on a membrane in a western blot using S-protein AP conjugate (Novagen Cat. No. 65958-3) or S-protein HRP conjugate (Novagen Cat. No. 69047-3)

Steps to detect protein marker along with the sample proteins on membrane:

1. Blot is incubated with primary antibody for the protein of interest
2. Incubate with secondary antibody – e.g. anti-mouse (or any other species - goat, sheep etc) IG-AP conjugate
3. Include S-Protein AP or HRP conjugate with the secondary antibody incubation
4. Process using chemiluminescent detection for alkaline phosphatase

*Note: Markers can also be visualized in gel with SYPRO® Tangerine Gel Stain before Western blotting without inhibition of protein transfer.*