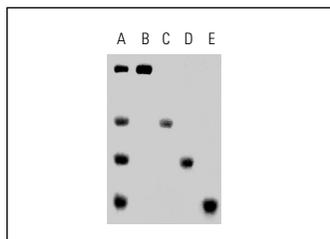


High Range Markers: sc-2362

PRODUCT

Santa Cruz Biotechnology, Inc. offers High Range Markers for use as pre-stained molecular weight standards in Western blotting applications. The ladder consists of four bands (unstained molecular weights). Please see the sticker below for stained molecular weights of the current lot. Each vial contains 500 μ l, sufficient product for 100-500 uses. Protein markers are listed below.



High Range Markers: sc-2362. SDS-PAGE analysis of High Range Markers, showing total marker panel (A), and individual 200 kDa (B), 97 kDa (C), 66 kDa (D) and 44 kDa (E) protein markers.

PROTEIN MARKERS

Unstained Protein MW

200 kDa
97 kDa
66 kDa
44 kDa

PROCEDURE

Broad Range Markers are provided in SDS-PAGE loading buffer and may be loaded directly into an SDS-PAGE gel. Allow markers to come to room temperature before use. Load 1-5 μ l per lane in a mini-gel system. Sample preparation procedures are provided for monolayer cells, suspension cells and tissue samples. Follow the procedure suited to your needs.

ELECTROPHORESIS

- Mix sample (40–60 μ g whole cell lysate, 10–20 μ g nuclear extract, 5-10 μ g transfected lysate, or 10–20 ng purified protein per lane) with an equal volume of 2x electrophoresis sample buffer (sc-24945) and boil for 2–3 minutes. Unused samples may be stored at -20° C.
- Load up to 10 μ l of lysate per 1.0 mm of well width for gels of 0.75 mm thickness.
- We recommend the use of Cruz Marker™ molecular weight standards (sc-2035). Load 2 μ l/well for 0.75 mm gels and 5 μ l/well for 1.5 mm gels. When used with Cruz Marker™ compatible mouse IgG binding proteins, internal standard bands will appear when the probed blot is exposed to detection reagent. Alternatively, use Prestained Molecular Weight Standards (sc-2361).
- Electrophorese according to standard protocols.
- Transfer proteins from the gel to a nitrocellulose or PVDF membrane using an electroblotting apparatus according to the manufacturer's protocols.

IMMUNOBLOTTING

- Block non-specific binding by incubating membrane in Blotto (either TBS Blotto A: sc-2333 or TBS Blotto B: sc-2335) for 30–60 minutes at room temperature (UltraCruz® Gel Incubation Trays in multiple colors and 200 ml or 120 ml sizes available). Alternatively, the membrane may be blocked at 4° C overnight in a covered container, using Blotto without Tween-20.
- If using a phospho-specific antibody, add 0.01% (v/v) of each Phosphatase Inhibitor Cocktails A and B (sc-45044 and sc-45045) to the blocking solution and the antibody diluent to inhibit phosphatases.
- Incubate the blocked membrane in primary antibody diluted in Blotto for 1 hour at room temperature. (For phospho-specific antibodies: Use Blotto B with 0.01% (v/v) of each Phosphatase Inhibitor Cocktails A and B (sc-45044 and sc-45045.) Optimal antibody concentration should be determined by titration. We recommend a starting dilution of 0.5-2.0 μ g/ml. Wash membrane three times for 5 minutes each with TBST.

IMMUNOBLOTTING – HORSE RADISH PEROXIDASE (HRP) DETECTION

- Incubate the membrane for 45 minutes at room temperature with horseradish peroxidase (HRP) conjugated secondary antibody, or mouse IgG binding protein (m-IgG κ -BP-HRP: sc-516102, or m-IgG λ -BP-HRP: sc-516132), diluted to 1:500-1:2000 in Blotto. If high backgrounds are observed, secondary antibody or detection reagent should be diluted further (up to 1:20,000). If Cruz Marker™ molecular weight standards (sc-2035) are used in the gel, the Cruz Marker™ compatible mouse IgG binding proteins (sc-516102-CM or sc-516132-CM) must be used in order to visualize standards with ECL.
- Wash membrane three times for 5 minutes each with TBST and once for 5 minutes with TBS.
- Incubate membrane in Chemiluminescence Luminol Reagent (sc-2048) according to Luminol datasheet, or visualize proteins using standard protocols. If luminol is used for visualization, an HRP-conjugated secondary antibody or mouse IgG binding protein must be used.

STORAGE

Store vial of Low Range Markers at -20° C.

STAINED MOLECULAR WEIGHTS OF CURRENT LOT



RESEARCH USE

For research use only, not for use in diagnostic procedures.