SANTA CRUZ BIOTECHNOLOGY, INC.

Broad Range Markers: sc-2361

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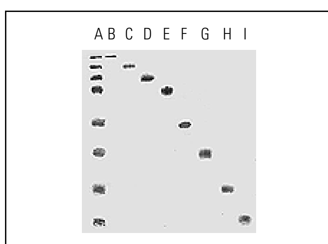
The Power to Question

PRODUCT

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



Broad Range Markers: sc-2361. SDS-PAGE analysis of Broad Range Markers, showing total marker panel (**A**), and individual 200 kDa myosin (**B**), 97 kDa phosphorylase b (**C**), 66 kDa BSA (**D**), 44 kDa ovalbumin (**E**), 29 kDa carbonic anhydrase (**F**), 17 kDa myoglobin (**G**), 14 kDa lysozyme (**H**) and 6 kDa aprotinin (**I**) markers.

PROTEIN MARKERS

Protein	Unstained MW
myosin	200 kDa
phosphorylase b	97 kDa
BSA	66 kDa
ovalbumin	44 kDa
carbonic anhydrase	29 kDa
myoglobin	17 kDa
lysozyme	14 kDa
aprotinin	6 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 µl 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.

MONOLAYER CELLS

- Grow cells to subconfluency in a 100 mm x 20 mm petri dish, remove culture medium and rinse cell monolayer with room temperature 1x PBS (10X liquid PBS: sc-24946). The following steps should be performed on ice or at 4° C using fresh, ice cold buffers.
- Add 0.6 ml of RIPA buffer (sc-24948) to the monolayer cells in the plate. Gently rock plate for 15 minutes at 4° C. Remove adherent cells with a cell scraper. Transfer the resulting lysate to a microcentrifuge tube.
- Wash plate once with 0.3 ml of RIPA buffer and combine with first lysate. (Optional: Add 10 µl of 10 mg/ml PMSF (sc-3597) stock and/or pass through a 21-gauge needle to shear the DNA.) Incubate 30-60 minutes on ice.
- Centrifuge cell lysate at 10,000xg for 10 minutes at 4° C. The supernatant fluid is the total cell lysate. Transfer the supernatant to a new microcentrifuge tube. This is your whole cell lysate. For increased protein recovery, resuspend the pellet in a small volume of RIPA, centrifuge and combine supernatants.

SUSPENSION CELLS

- Collect ± 2.0 x 10^7 cells by low-speed centrifugation (e.g. 200xg) at room temperature for 5 minutes. Carefully remove culture medium.
- Wash the pellet with PBS at room temperature, and again collect by low-speed centrifugation. Carefully remove supernatant.
- Add 1.0 ml of ice cold RIPA buffer (sc-24948) with freshly added protease inhibitors and/or phosphatase inhibitors. Gently resuspend cells in RIPA buffer with a pipet and incubate on ice for 30 minutes.
- Further disrupt and homogenize cells by hydrodynamic shearing (21-gauge needle), dounce homogenization or sonication, taking care not to raise the temperature of the lysate. (Optional: Add 10 µl of 10 mg/ml PMSF stock (sc-3597). Incubate 30 minutes on ice.
- Transfer to microcentrifuge tube(s) and centrifuge at 10,000xg for 10 minutes at 4° C. The supernatant fluid is the total cell lysate. Transfer the supernatant to a new microfuge tube. This is your whole cell lysate. For increased protein recovery, resuspend the pellet in a small volume of RIPA, centrifuge and combine supernatants.

TISSUE SAMPLES

- Weigh tissue and dice into very small pieces using a clean razor blade. Frozen tissue should be sliced very thinly and thawed in RIPA buffer (sc-24948) containing protease inhibitors and/or phosphatase inhibitors. Use 3 ml of ice cold RIPA buffer per gram of tissue.
- Further disrupt and homogenize tissue with a dounce homogenizer or a sonicator, maintaining temperature at 4° C throughout all procedures. (Optional: Add 30 µl of 10 mg/ml PMSF (sc-3597) stock per gram of tissue.) Incubate on ice for 30 minutes.
- Transfer to microcentrifuge tubes, centrifuge at 10,000xg for 10 minutes at 4° C. Remove supernatant and centrifuge again. The supernatant fluid is the total cell lysate. A longer centrifugation may be necessary to obtain a clear lysate.

STORAGE

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

STAINED MOLECULAR WEIGHTS OF CURRENT LOT

RESEARCH USE

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