



PRODUCT DESCRIPTION

FOR LAB RESEARCH USE ONLY
NOT FOR USE IN HUMANS OR ANIMALS

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Protocol for Western Blot

Preparation of Cell Lysate:

Add RIPA buffer to cells (100ul to a 35 mm dish, 200ul to a 60 mm dish, 500ul to a 100 mm dish) while the culture dish is placed on ice. Scrape the cells and gently rock the suspension on either a rocker or an orbital shaker in the cold room for 15 minutes to lyse cells. Sonicate in ice water with bath sonicator, until the sample is no longer viscous. Centrifuge the cells at 12000g at 4°C for 5 minutes to remove pellet. Move the supernatant to a fresh tube. Final concentration of cell lysate will be 2-3ug/ul. Add 5 x SDS stop buffer to the lysate to a 1xSDS final concentration.

Immunoblotting:

1. After boiling for 10 minutes, 50-80ul sample will be loaded to SDS PAGE. For most proteins, **100-200ug total lysate proteins per lane** loading amount is suggested, because 80% of protein species in cells are at very low concentration.
2. Soak the gel in western blot transfer buffer. Cut a piece of membrane to the size of the gel. **PVDF membrane is recommended for most proteins.** Dip the membrane into methanol for 1-2 minutes, soak the membrane in transfer buffer for 10 minutes, and place it on a thick stack of buffer-soaked filter paper. Then cover it with another stack of buffer soaked filter papers. Cover up the transfer apparatus. Gel should be on the negative side of the membrane.
3. Run for 90 minutes at current of 1mA per cm².
4. Incubate the membrane in blocking buffer for 1 hour at room temperature or overnight at 4°C. Wash membrane 3x5min with washing buffer.
5. Dilute primary antibody with blocking buffer. Incubate the membrane for 1 hour at room temperature or overnight at 4°C. Wash membrane 3x5min with washing buffer.
6. Dilute secondary antibody with blocking buffer. Incubate the membrane for 1 hour at room temperature. Wash membrane 3x5min with washing buffer.
7. Wash membrane 3x10min with washing buffer.
8. Develop color with ECL.