Western Blot Protocol

Complete Protocol from Cell Lysis to Band Visualization

← All Protocols

Overview

Western blotting (immunoblotting) detects specific proteins in cell or tissue lysates through protein separation by size, transfer to membrane, and antibody-based detection. The complete procedure takes approximately 2-3 days.

SAFETY

RIPA buffer contains SDS and detergents. β-mercaptoethanol is toxic with strong odor. Acrylamide is a neurotoxin. Always wear gloves, lab coat, and safety glasses. Work in fume hood when handling β-ME. Consult Safety Data Sheets before use.

Day 0: Preparation

- Prepare inhibitor stocks
- Make base RIPA buffer
- Prepare working buffers

Day 1: Lysis & Transfer

- Cell lysis & BCA assay
- Cast and run gels
- Overnight protein transfer

Day 2: Antibodies & Detection

- Block membrane
- Primary & secondary antibodies
- Detection and imaging

Sample Requirements

Sample Type	RIPA Buffer Volume	Expected Yield
6-well plate (1 well, 80-100% confluent)	60-80 µL	100-300 µg
60 mm dish (80-100% confluent)	100-200 µL	200-500 µg
100 mm dish (80-100% confluent)	300-500 µL	500-1500 µg