

## Western Blotting

---

### Reagents

**-2x Sample buffer**

125mM Tris-HCl (pH6.8), 4% SDS, 20% Glycerol, 10% 2-Mercaptoethanol, 0.02% BPB

**-HRP conjugated secondary antibody**

Anti-Rabbit IgG (H+L) Goat IgG Fab' HRP (IBL, #17502) or

Anti-Mouse IgG (H+L) Goat IgG Fab' HRP (IBL, #17601)

**-Blocking solution**

3% milk, 1% BSA, 0.05% NaN<sub>3</sub> / PBS

**-Washing solution**

0.05% Tween20/PBS

**-ECL Western Blotting Detection Reagent**

GE Healthcare, # RPN2106

### Procedure

1. **PAGE:** Apply 10 - 20μL of prepared sample to polyacrylamide gel (7-12 %).
2. **Electrophoresis**
3. **Blotting:** Transfer to a nylon membrane.
4. **Blocking the membrane with blocking solution:** 2hrs. at 37°C
5. **Wash with washing solution, 5 min. x 3 times**
6. **Primary antibody:** 2hrs. at 37°C or overnight at 4°C
7. **Wash with washing solution, 5 min. x 3 times**
8. **Second antibody:** 1hr. at 37°C
9. **Wash with washing solution, 5 min. x 3 times**
10. **Detection with ECL**

### Example of sample preparation

**Cell lysate**

- 1) **Wash the cultured cells with PBS and trypsin treatment as necessary.**
- 2) **Wash with PBS after cessation of trypsin action and count the number of cells.**
- 3) **Suspend the cells in 2x Sample buffer (1 - 5 x 10<sup>5</sup> cells/10μL).**
- 4) **Sonication**
- 5) **Boiling (heat block), 3min.**
- 6) **Centrifugation, 14,000 rpm. 3min. at 4°C**
- 7) **Use the supernatant.**

**Cell culture medium**

**Use the supernatant**