Protocol

<Advanced Course >

A. Western blotting

1. Proteins are separated by SDS-PAGE (see Core curriculum B. Protein)
2. After electrophoresis, equilibrate the gels in transfer buffer
3. Transfer proteins electrophoretically to polyvinyliden fluoride (PVDF) membranes
4. Block non-specific binding sites by immersing the membrane in 5% fat-free skim milk in PBS containing 0.005% Tween 20 for 30-60 min at RT
5. Wash with TBS-T 3 times
6. Incubate membranes with appropriately diluted primary antibody* for 40-60min at RT
7. Wash with TBS-T 3 times
8. Incubate membranes with diluted HRP-conjugated secondary antibody** for 40-60 min at RT
9. Wash with TBS-T 3 times and incubate the membrane in the dilution for 30 min at RT on an orbital shaker
10. Drain the excess wash buffer and put the membrane in Ziploc
11. Mix detection solutions A and B in a ratio of 40:1 and pipette it in Ziploc
12. Gently smooth out any air bubbles and place the membrane in chemiluminescence detector.

*We use p44/p42 Map Kinase (Erk1/Erk2) antibody and Pospho-p44/p42 Map Kinase (Thr202/Tyr204) antibody for primary antibody.

**We use HRP-conjugated anti-rabbit IgG whole antibody for secondary antibody.

Pospho-p44/p42 MapK Ab