Lysate Preparation Protocol

To lysate cell

WB lysate preparation (RIPA)

a. Discard the medium in the flask and wash once with pre-cold PBS.

b. Add 3 ml pre-cold PBS per flask and collect cells with cell scraper.

c. Add 12 ml pre-cold PBS to make sure all the cells detach from the flask.

d. Transfer collected cells to 50 ml centrifuge tubes, centrifuge with 1200 ~3000 rpm, 5 ~10 min.

e. Discard supernatant, wash twice with pre-cold PBS.

f. Add RIPA buffer according to the cell amount to re-suspend cells (place on ice for 15
min).

g. Use ultrasonic cell disruptor to break all cell clusters until the lysate becomes clear. (Ultrasound time 3 s, 10 s interval, ultrasonic 5 ~15 times)

h. Centrifuge for 5~10 minutes at 15000 ~17000 g and discard cell pellet.

To lysate tissue

WB lysate preparation (RIPA)

a. To shatter the frozen tissue with pre-cold scissor, to grind tissue into powder with a tissue grinding instrument.

b. Add RIPA buffer according to the tissue amount to re-suspend tissue (place on ice for
15 min).

c. Use ultrasonic cell disruptor to break all cell clusters until the lysate becomes clear.

d. Centrifuge for 5~10 minutes at 15000 ~17000 g and discard tissue pellet.

2× Sample Buffer

- 125 mM Tris-HCl (pH 6.8)
- 2.5% SDS
- 0.04% Bromophenol Blue
- 25% Glycerol:
- 01mM DTT
- ddH_2O