Lysate Preparation Protocol

To lyse the cell

WB 1%SDS Hot Lysate buffer preparation

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 min.

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

f. Heat 1%SDS Hot lysis until bubbling.

g. Add 1%SDS Hot cell lysis according to the cell amount to re-suspend cells (pipetting in boiling water for 10 ~20 min).

h. 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, ultrasonic power: 40 kW)

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

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 ~10min.

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, ultrasonic power: 40 kW)

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

To lysate tissue

WB 1%SDS Hot Lysate buffer preparation

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

pre-cold mortar.

b. Heat 1%SDS Hot lysis until bubbling.

c. Add 1%SDS Hot cell lysis according to the tissue amount to re-suspend cells (pipetting in boiling water for 10 ~ 20 min).

d. Use ultrasonic cell disruptor to break all cell clusters until the lysate becomes clear. (Ultrasound time 3s, 10s interval, ultrasonic 5 ~15 times, ultrasonic power: 40 kW)

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

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. (Ultrasound time 3 s, 10 s interval, ultrasonic 5 ~15 times, ultrasonic power: 40 kW)

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

1×1%SDS Hot Lysate buffer

- 10 mM Tris-Hcl (pH8.0)
- 1%SDS
- 1.0 mM Na-Orthovanadate
- ddH_2O

2×Sample Buffer

- 62.5mM Tris-Hcl (pH6.8)
- 2% SDS
- 0.01% Bromophenol Blue
- 25% Glycerol:
- 710mM ß-Mercaptoethanol:
- ddH_2O