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 ~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, 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
- mM Na-Orthovanadate
- ddH$_2$O

**2×Sample Buffer**

- 62.5mM Tris-Hcl (pH6.8)
- 2% SDS
- 0.01% Bromophenol Blue
- 25% Glycerol:
- 710mM ß-Mercaptoethanol:
- ddH$_2$O