1%SDS Hot Lysates Preparation

To lyse the cell

1. Discard the medium in the flask and wash once with pre-cold PBS.
2. Add 3ml of pre-cold PBS per flask. Detach the cells from the flask with cell scraper.
3. Add 12ml of pre-cold PBS to re-suspend the detached cells.
4. Centrifuge and collect the cells at 100g-500g for 5min. Wash the cells twice with pre-cold PBS.
5. Heat 1%SDS hot lysis buffer to 90-95℃. Re-suspend the cells with the buffer.
6. Pipetting the cells in boiling buffer for 1 minute. Then boil them at 90-95℃ for 10-20 min. (Mix the samples periodically during the boiling)
7. Sonicate the cells (40kW, 3 seconds, intervals 3 seconds, 25-30 times) until the cell clumps scatter and the liquid is clear.
8. Centrifuge for 5-10 minutes at 15000-17000g and discard the cells.
9. Protein concentration quantitative analysis by BCA method for the lysates.
10. Adjust the concentration of the lysates to 4 mg/ml with 1%SDS hot lysis buffer.
11. Add 2 x loading buffer to make the final lysates concentration of 2mg/ml.
12. Boil the lysates for 5 minutes. Then ready to use.
13. Aliquot and store the lysates at -20°C or -80°C for long term use. Boil the lysates for 5 minutes before sample loading.

To lyse the tissue

1. Cut the frozen tissue into small pieces with scissors.
2. Homogenate the tissues with homogenizer, or grind the small piece using mortar and pestle. (The scissors, mortar and pestle should be pre-chilled at -80°C overnight.)
3. Transfer the tissues to a pre-chilled centrifuge tube.

For the following steps, please see 5-12 in "To lyse the cells".

Solution preparation

1. 1%SDS hot lysis buffer

10 mM Tris-Hcl (pH8.0)

1%SDS

1.0 mM Na-Orthovanadate

ddH_2O

2. 2 x loading buffer

62.5mM Tris-Hcl (pH6.8)

2% SDS

0.01% Bromophenol Blue

25% Glycerol:

710mM ß-Mercaptoethanol:

ddH2O