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 1200-1500 rpm for 5 minutes. Wash the cells twice with pre-cold PBS.
5. Heat 1%SDS hot lysis buffer until boiled. Re-suspend the cells with the buffer.
6. Pipette the cells in boiling buffer for 1 minute. Then boil them for 10-20 minutes. (Mix the samples periodically during the boiling)
7. Sonicate the cells (3 seconds, intervals 3 seconds, 25-30 times) until the cell clumps scatter and the liquid is clear.
8. Centrifuge 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 tShe 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×Sample Buffer

125mM Tris-Hcl (pH6.8)
2.5% SDS

0.04% Bromophenol Blue

25% Glycerol:

01mM DTT

ddH2O