Total Lysis Buffer

CatNo:TLB 001

for Western blotting and 2D gel electrophoresis

Store at 4°C.

Feture: Protease inhibitors or phosphatase inhibitors are not needed in this lysis buffer. Most membrane proteins are extracted with high efficiency.

Stock solution: 25 ml, ** (使用前務必請先回溫至室溫)

Stock powder: 25 grams

Working solution: 1 g stock powder + 1.0 ml stock solution'gently mixing at 37 °C, centrifuged at 10,000 rpm for 10 min at room temperature to remove trace of undissolved substance.

Table 1. Volume of lysis buffer needed for cultured cells

Growth area	Volume of culture media	Volume of lysis buffer
12.5 cm^2	4.0-5.0 ml	0.25-0.30 ml
25 cm^2	8.0-9.0 ml	0.50-0.70 ml
75 cm^2	20-30 ml	0.80-1.0 ml

Procedures

1.: *for lysis of cultured cells*

1. Add appropriate volume of lysis buffer into the culture dish.
2. Use Policeman to scratch cells carefully at room temperature
3. Collect lysate and centrifuge at 10,000 rpm for 10 min at room temperature.
4. Collect supernatant and store the sample at 4 °C.

5-1 Western blotting: Mix with an equal volume of 2X SDS sample buffer and use the mixture for SDS-PAGE.

5-2. Isoelectric focusing: Take 100 ul of lysate, add 100 ul of 20% TCA, mix for a few seconds. Add 1.0 ml cold acetone and mix for a few seconds. Keep the mixture at -20 °C overnight. Centrifuge at 10,000 rpm for 10 min at 4 °C, remove all supernant carefully. Add 1.0 ml cold acetone and mix for a few seconds. Centrifuge at 10,000 rpm for 10 min at 4 °C, remove all supernant carefully. Keep the pellet and redissolve the pellet with IEF rehydration buffer. Centrifuge to remove undissolved substances if necessary.

2.: *for lysis of animal tissues*

1. Add 10X volume of lysis buffer to the tissue.
2. Use scissors to dissect the tissues into smaller pieces.
3. Homogenize the tissues with a glass homogenizer or with a Polytron device.
4. Centrifuge at 10,000 rpm for 10 min at room temperature.
5. Collect supernatant and store the sample at 4 °C.

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