PRODUCT INFORMATION

Mammalian Protein Extraction Kit (Cat# PEM-1000)

Production Information for PEM-1000:

Component:

Component	For 5x (10_7-10_8) cells or 50 plates (100 mm) or 1g tissue
5X TE Buffer,	10 ml
NaCl Solution	10 ml
SDS Solution	10 ml
DOC Solution	10 ml
0.5% NP-40 Solution	10 ml
20x PBS	10 ml
Protease Inhibitor Cocktail	0.5 ml

Procedure:

1 The Cell Lysis Buffer is prepared by mixing the following components, then 10 µl of Protease Inhibitor Cocktail is added to the mix.

5X TE Buffer,	200 µl
0.75M NaCl	200 µl
0.5% SDS	200 µl
2.5% DOC	200 µl
0.5% NP-40	200 µl
Total Volume	1.0 ml

2 Wash cells/ tissue and then treat with 1ml of the Cell Lysis Buffer.

a) For adherent cells: Remove the growth media from the cells to be assayed. Rinse the cells twice with 1x PBS carefully, add Cell Lysis Buffer (10^6-10^7 cells/ml); incubating for 5 minutes on a shaker; scrape and collect cells.

b) For cells in suspension: Centrifuge for 5 minutes at 420 x g. Decant the supernatant. Wash the cells twice by resuspending the pellets in 1x PBS; centrifuge and discard the supernatant. Re-suspend the pellet in Cell Lysis Buffer (10^6-10^7 cells/ml); incubate for 5 minutes.

c) For tissues: Rinse the tissue twice with 1x PBS. Add Cell Lysis Buffer (5-20 mg tissue/ml); incubate for 5 minutes; transfer the sample (with Cell Lysis Buffer) to a pre-chilled micro-homogenizer and homogenize the tissue. Be aware that homogenization procedure is critical for the functional integrity of the target protein.

3. Centrifuge the lysed cells for 10 minutes at 12,000 x g to pellet the cellular debris. Alternatively, to prepare a protein solution using high-speed centrifugation, centrifuge for 45 minutes at 100,000 x g. Remove the protein-containing supernatant to a chilled tube. Keep on ice for immediate use, or store it at -70°C.

Note:

1 In special cases when a concentrated lysate is required, the cells can be lysed using a lower volume of cell lysis buffer. For adherent cells, the plate size will dictate the amount of buffer covering the plate surface. For cells in suspension, the volume can be decreased to a volume of 2x volume of packed cells.

2 Some antigens can be denatured and some protein:protein complexes can be disrupted in the presence of the complete Cell Lysis Buffer containing all three detergents. In such cases, the suitable detergent(s) must be carefully chosen. If one or two of the detergent solutions are to be omitted, use an equal volume of distilled deionized water to replace the detergent solution(s).

3 The Protease Inhibitor Cocktail is properly used at a 1:100 dilution in the cell lysis buffer.

4 Perform all steps at 2-8°C after preparation of the Cell Lysis Buffer.

Caution:

Operate carefully with PMSF. It is extremely toxic and is irritating to eyes and skin. Wear eye and hand protection to keep the solution containing PMSF away from skin and eyes. This product is for laboratory research use only.

Storage:

Store Protease Inhibitor Cocktail at -20^0 C, and keep the rest of components at room temperature.

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