Protein Sample Preparation

Protocol

Bulletin 6195

Human Cells

Use the MicroRotofor™ cell lysis kit (mammalian) or the protocol detailed here, which uses sonication and radioimmunoprecipitation assay (RIPA) buffer, for cell lysis and protein extraction.

Suspension Cultured Cells

1. Pellet the cells by centrifugation at 2,000 × g for 5 min at 4°C.
2. Discard the supernatant and wash pelleted cells in cold PBS. Repeat steps 1 and 2 twice.
3. Add RIPA buffer to the pelleted cells and suspend the pellet with a pipet.

Monolayer Cultured Cells

1. Carefully remove (decant) culture medium from cells. Wash cells twice in cold PBS.
2. Add RIPA buffer to the cells and keep on ice for 5 min. Swirl the plate occasionally to spread the buffer around the plate.
3. Use a cell scraper to collect the lysate and transfer to a microcentrifuge tube.

4. Place the cell suspension on ice, incubate 5 min, and sonicate at appropriate intervals. Check lysis efficacy by light microscopy.
5. Centrifuge cell debris at ~14,000 × g for 15 min at 4°C and transfer supernatant to a new vial.
6. Perform a protein assay of the supernatant. A protein concentration of 3–5 µg/µl is best for PAGE.
7. Add 2x SDS-PAGE sample buffer to the protein solution to yield a 1× sample buffer concentration.

REAGENTS

- Phosphate buffered saline (PBS)
- RIPA solubilization buffer (use 1 ml RIPA buffer with 3×10^7 cells; store and use RIPA buffer at 4°C
- SDS-PAGE sample buffer (2×)

EQUIPMENT

- Centrifuge
- Sonicator

PRODUCT LINKS

- MicroRotofor cell lysis kit (mammalian)
- *RC DC* protein assay
- SDS-PAGE sample buffer (2×)

BIO-RAD

This is an excerpt from Bio-Rad's comprehensive Electrophoresis Guide (Bulletin 6640).

Bio-Rad Laboratories, Inc.

Life Science Group

Web site *www.bio-rad.com* ***USA*** *800 424 6723* ***Australia*** *61 2 9914 2800* ***Austria*** *01 877 89 01* ***Belgium*** *09 385 55 11* ***Brazil*** *55 11 5044 5699*
Canada *905 364 3435* ***China*** *86 21 6169 8500* ***Czech Republic*** *420 241 430 532* ***Denmark*** *44 52 10 00* ***Finland*** *09 804 22 00*
France *01 47 95 69 65* ***Germany*** *089 31 884 0* ***Greece*** *30 210 9532 220* ***Hong Kong*** *852 2789 3300* ***Hungary*** *36 1 459 6100* ***India*** *91 124 4029300*
Israel *03 963 6050* ***Italy*** *39 02 216091* ***Japan*** *03 6361 7000* ***Korea*** *82 2 3473 4460* ***Mexico*** *52 555 488 7670* ***The Netherlands*** *0318 540666*
New Zealand *64 9 415 2280* ***Norway*** *23 38 41 30* ***Poland*** *48 22 331 99 99* ***Portugal*** *351 21 472 7700* ***Russia*** *7 495 721 14 04*
Singapore *65 6415 3188* ***South Africa*** *27 861 246 723* ***Spain*** *34 91 590 5200* ***Sweden*** *08 555 12700* ***Switzerland*** *061 717 95 55*
Taiwan *886 2 2578 7189* ***Thailand*** *800 88 22 88* ***United Kingdom*** *020 8328 2000*

Bulletin 6195 Rev A US/EG *11-0864 1111 Sig 1211*