User Manual

Whole Cell Extraction Kit

2910

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures. Not for human or animal consumption.

Product Overview

The Chemicon®'s Whole Cell Extraction Kit (2910) provides a simple and convenient method for the preparation of whole cell extractions for use in experimental studies of various cellular proteins. This kit is recommended for use with Rac1 Activation Magnetic Beads Pulldown Assay (17-10393), Cdc42 Activation Assay Kit (17-286), Ras Activation Assay Kit (17-218), and Chemicon®'s Transcription Factor Assays when purified nuclear extracts are not required.

Materials Provided

- Whole Cell Extraction Buffer: 5X (90493) Two vials containing 10 mL of 5X concentrated buffer. Dilute to 1X final concentration with double distilled H_2O prior to use.
- Protease Inhibitor Cocktail: (90492) One vial containing 100 µL of protease inhibitors in DMSO for use with mammalian cells and tissue extracts. A mixture of protease inhibitors with broad specificity for the inhibition of serine, cysteine, and aspartic acid proteases and aminopeptidases. Contains 4-(2-aminoethyl)benzenesulfonyl fluoride (AEBSF), pepstatin A, E-64, bestatin, leupeptin, and aprotinin. Contains no metal chelators. Prior to use, dilute 1/1000 in 1X Whole Cell Extraction Buffer.

Materials Required (Not supplied)

- Cultured Cells and Tissue Culture Supplies
- Cell Detachment Buffer (Trypsin) or Cell Scrapers
- Phosphatase Inhibitors (if desired)
- Syringes, 1 mL, 27 - Gauge Needle
- 1.5 mL Microcentrifuge tubes
- Deionized Water
- Table-top Microcentrifuge, 4 °C
- Phosphate-Buffered Saline (PBS), Tris-Buffered Saline (TBS), or HBSS (Hank's Balanced Salt Solution)

Storage and Stability

- The 5X Whole Cell Extraction Buffer can be stored at 2-8 °C until expiration date.
- The prepared 1X Whole Cell Extraction Buffer (with optional additives) should be stored at –20 °C for up to one month.
- The undiluted Protease Inhibitor Cocktail can be stored at –20 °C until the expiration date.

Protocol

Preparation of Reagents

5X Whole Cell Extraction Buffer

Dilute to 1X by adding 4 mL of distilled water to each 1 mL of 5X Whole Cell Extraction Buffer. Prior to using the diluted buffer, add Protease Inhibitor Cocktail and additional phosphatase inhibitors, if desired. Equilibrate on ice prior to use.

Preparation of Whole Cell Extraction

1.0 mL	Whole Cell Extraction Buffer (1X)
1.0 µL	Protease Inhibitor Cocktail
1.001 mL	Total volume of buffer per $1x10^7$ cells or per T75 flask

Extraction Procedure

Preparation of Lysates from Adherent Cells

1. Culture cells to ~ 70-90% confluency.
2. If necessary, treat cells with desired method.
3. Aspirate culture media and wash cells twice with ice-cold Phosphate-Buffered Saline (PBS), Tris-Buffered Saline (TBS), or HBSS.
4. Dissociate cells from culture flask by either the addition of trypsin to the adherent cell culture flask allowing cells to dissociate from the flask (approximately 2-5 minutes, depending on cell type) or by using a cell scraper. Choose method appropriate for desired protein.
5. Transfer cell suspension to an appropriate container and wash two times with two volumes of ice-cold PBS. Pellet by spinning at 250 x *g* for 5 minutes at 4 °C.
6. Continue to section Preparation of Lysates from Non-Adherent Cells, step 5.

Preparation of Lysates from Non-Adherent Cells

1. Culture cells to desired cell density.
2. Count cells and pellet by centrifugation at 250 x *g* for 5 minutes at 4 °C.
3. Following centrifugation, rinse cell pellet twice with ice-cold 1X PBS or 1X TBS.
4. Centrifuge and decant supernatant.
5. While on ice, gently resuspend cell pellet by the addition of ice-cold 1x Whole Cell Extraction Buffer with Protease Inhibitors (approximately 0.8 mL per 10 cm dish or 1 mL per T75 flask).
6. Gently draw the solution through a 27-gauge needle 5 times; avoid producing bubbles.
7. Incubate on ice for 15 minutes.
8. Centrifuge at 8,000 x *g* (table-top microcentrifuge at 13K RPM) for 20 minutes at 4 °C.
9. Collect the supernatant which contains cytosolic and nuclear proteins. For membrane bound proteins, do not centrifuge and pellet the lysate. Aliquot and place on ice for immediate use, or snap freeze and store at -70 °C for future use.
10. Protein concentration may be determined following collection of the desired protein fraction prior to freezing. Adjust the concentration as desired using ice cold PBS, TBS, or 1X Whole Cell Extraction Buffer containing protease inhibitors.

Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

Technical Assistance

Visit the tech service page at SigmaAldrich.com/techservice.

Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at SigmaAldrich.com/terms.

Contact Information

For the location of the office nearest you, go to SigmaAldrich.com/offices.

Document Template 00007539FM, Ver 13.0

2910 Ver 4.0, Rev 06JUN2024, DP