ab288085 – Fraction-PREP Cell Fractionation Kit

For serial sample preparation of four distinct protein fractions including cytosol/particulate/cytoskeleton/nuclear fractions, from one sample
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab288085

Storage and Stability

On receipt entire assay kit can be stored at -20°C. After opening the kit, you may store buffers at +4°C or –20°C. Store Protease Inhibitor Cocktail I and DTT STOCK (1M) at –20°C. Use kit within 12 months.

Materials Supplied

Item	Quantity	Storage Condition
Extraction Buffer III	25 ml	-20°C
Extraction Buffer VI	20 ml	-20°C
Lysis Buffer X	1.2 ml	-20°C
Extraction Buffer V	10 ml	-20°C
DTT STOCK (1M)	150 µl	-20°C
Protease Inhibitor Cocktail I	1 vial*	-20°C

*Add 150 µl of DMSO, and mix well before use.

PLEASE NOTE: DTT Stock (1M) was previously labelled as DTT I and DTT (1 M). The composition has not changed.

General Consideration and Reagent Preparation:

- After opening the kit, you may store buffers at +4°C or –20°C. Store Protease Inhibitor Cocktail I and DTT STOCK (1M) at –20°C.
- Before starting the procedure, prepare sufficient Extraction Buffer Mix (EB-Mix) for your experiment: Add 2 µl Protease Inhibitor Cocktail I and 2 µl DTT STOCK (1M) to 1 ml of Extraction Buffer III, Extraction Buffer VI, and Extraction Buffer V, individually.
- Be sure to keep all buffers on ice at all times during the experiment. All centrifugation procedures are recommended to be performed at 4°C.
- The following protocol is described for fractionation of 4 - 8 x 10^6 cells. If more cells are used for fractionation, scale up the volumes proportionally.

Fractionation Protocol:

1. Collect cells (4 - 8 x 10^6) by centrifugation at 700 x g for 5 min. Wash cells with 5 - 10 ml of ice-cold PBS and centrifuge at 700 x g for 5 min. If using fresh tissue, cut the tissue (~400 mg) into small pieces, add ice cold PBS (1 - 2 ml), and homogenize in a manual tissue homogenizer. Pellet the cells by centrifugation at 500 x g for 5 minutes and remove the supernatant.
2. Resuspend the cell pellet in 1 ml of ice-cold PBS and transfer cells to a microfuge tube. Spin for 5 min at 700 x g and remove supernatant.
3. Resuspend the pellet in 400 µl of Extraction Buffer III Mix (EB-Mix containing DTT STOCK (1M) and Protease Inhibitor Cocktail I). Pipette several times to mix well with cells. Incubate sample on ice for 20 min with gentle tapping 3 - 4 times every 5 minutes.
4. Centrifuge the sample at 700 x g for 10 min. Collect supernatant (This is Cytosolic Fraction). Keep on ice.
5. Resuspend the pellet in 400 µl of ice-cold Extraction Buffer VI Mix (EB Mix containing DTT STOCK (1M) and Protease Inhibitor Cocktail I). Pipette several times and vortex the sample for 10 - 15 seconds to mix well.
6. Add 22 µl of Lysis Buffer X, vortex for 5 seconds. Incubate on ice for 1 min.
7. Vortex for 5 seconds again and centrifuge for 5 min at 1000 x g (3400 rpm).
8. Immediately transfer the supernatant to a clean pre-chilled tube (This is Membrane/Particulate Fraction). Keep on ice.
9. Resuspend the pellet in 200 µl of ice-cold Extraction Buffer V Mix (EB-Mix containing DTT STOCK (1M) and Protease Inhibitor Cocktail I), vortex for 15 seconds, keep on ice for 40 minutes with constant vortex for 15 seconds every 10 minutes.
10. Centrifuge the sample at top speed in a microcentrifuge for 10 minutes.
11. Transfer the supernatant to a clean pre-chilled tube (This is Nuclear Fraction). The pellet is the Cytoskeletal Fraction. The Cytoskeletal fraction can be dissolved in 100 µl of 0.2 % SDS containing 10 mM DTT STOCK (1M) or dissolve directly in SDS-PAGE sample buffer.
12. Store all fractions at –80°C for future use.

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)