PI-0106

CytoFrac Cell Fractionation Kit

(Sufficient for 50 isolations, Store at -20°C)

Introduction:

The CytoFrac Cell Fractionation system is designed to provide reproducible separation of four distinct subcellular fractions: Cytoplasmic, Nuclear, Subcellular Membrane/Particulate and Cytoskeletal fractions from a single mammalian sample. The procedure is fast and easy, requiring only 2 hours with no ultracentrifugation required. All four fractions obtained are suitable for diverse downstream applications such as 1 or 2D gel electrophoresis, enzyme activity assays, gel shift assay, and/or Western blotting.

Kit Components:

Cytosol Extraction Buffer (CEB)	20 ml	WM	PI-0106A
Membrane Extraction Buffer A (MEBA)	20 ml	WM	PI-0106B
Membrane Extraction Buffer B (MEBB)	1.2 ml	Green	PI-0106C
Nuclear Extraction Buffer (NEB)	10 ml	NM	PI-0106D
DTT (1 M)	150 µl	Blue	PI-0106E
Protease Inhibitor Cocktail (PIC)	lyoph	Red	PI-0106F

User Supplied Materials:

DMSO
PBS (ice cold)
0.2% SDS/10 mM DTT or SDS-PAGE Sample Buffer
Homogenizer (Dounce, Potter-Elvehjem or Polytron)

Storage and Handling Considerations:

The procedure is designed for a prep of 4 – 8 x 10^6 cells. If you plan to use a larger amount, scale up the volumes of buffers used, proportionately.
Store unopened kit at -20°C. Bring all components to room temperature before use. Centrifuge small vials briefly before opening.
Add 150 µl of DMSO to the Protease Inhibitor Cocktail, mix well and set aside.
Prepare Extraction Buffer Mixes by adding 2 µl of DTT and 2 µl of Protease Inhibitor Cocktail to 1 ml of CEB, to 1 ml of MEBA and to 1 ml of NEB.
Place the 3 buffers on ice, along with 3 empty tubes and keep them ice cold throughout the entire procedure. All centrifugations should also be performed at 4°C.
The buffers can all be stored at 4°C.
Store DTT and Protease Inhibitor Cocktail at -20°C.

Fractionation Protocol:

1. Collect 4 – 8 x 10^6 cells by gentle centrifugation at 700 X g for 5 minutes. Remove medium carefully and wash the cells with 5 – 10 ml of PBS, then centrifuge again at 700 X g and discard the supernatant.

   - If using tissue, mince the tissue (400-500 mg) into fine pieces in 1 – 2 ml of ice cold PBS and homogenize with a few passes.
   Centrifuge at 500 X g for 5 minutes to pellet the cells and discard the supernatant.
   Resuspend the cell pellet in 1 ml of ice cold PBS, transfer to a microfuge tube and centrifuge at 700 X g for 5 minutes. Discard the supernatant.
2. Resuspend the cell pellet in 400 µl of CEB containing DTT and PIC, prepared in advance above. Triturate the cells several time to mix thoroughly then place on ice for 20 minutes. Mix the tube gently by tapping a couple times every 5 minutes.
3. Centrifuge the lysed cells at 700 X g for 10 minutes. Carefully transfer the supernatant to a clean prechilled tube and place it on ice, without disturbing the pellet. This is the Cytosolic Fraction.
4. Add 400 µl of ice cold MEBA prepared in advance above. Triturate to mix and vortex for 10 – 15 seconds.
5. Add 22 µl of MEBB, vortex for 5 seconds, place on ice for 1 minute then vortex again for 5 seconds.
6. Centrifuge at 1000 X g for 5 minutes.
7. Immediately transfer the supernatant to a clean prechilled tube. This is the Membrane/Particulate Fraction. Place it on ice.
8. Add 200 µl of ice cold NEB prepared in advance above. Resuspend by triturating and vortex for 15 seconds. Place on ice for 40 minutes, vortexing for 15 seconds every 10 minutes.
9. Centrifuge at top speed ~ 16,000 X g for 10 minutes
10. Transfer the supernatant to a clean pre-chilled tube without disturbing the pellet. This is the Nuclear Fraction. Place it on ice.
11. The pellet is the Cytoskeletal Fraction. It can be dissolved in 0.2% SDS/10 mM DTT or directly in SDS-PAGE Sample Buffer.
12. Store all fractions at -80°C for future analysis.

FOR RESEARCH USE ONLY! Not to be used for diagnostic or therapeutic purposes.

48511 Warm Springs Blvd. # 213, Fremont, CA 94539 +1(408)739-9315 contact@akrivisbio.com