



Mitochondria/Cytosol Fractionation Kit

Manufactured by BioVision.

ALX-850-276-KI01

~100 tests

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For laboratory use only. Not for human or diagnostic use.

I. Introduction:

The **Mitochondria/Cytosol Fractionation Kit** provides unique formulations of reagents for effective isolation of a highly enriched mitochondrial fraction from cytosolic fraction of mammalian cells including both apoptotic and non-apoptotic cells. The enriched mitochondrial and cytosolic fractions can be used for studying apoptotic and signal transduction pathways to detect translocation of any factors interested between the two fractions by Western blotting, ELISA, or other assays. The cell fractionation procedure is so simple and easy to perform. No ultracentrifugation is required and no toxic chemicals are involved.

II. Kit Contents:

Components	850-276-KI01
	100 tests
Mitochondria Extraction Buffer	10 ml
5x Cytosol Extraction Buffer	20 ml
DTT (1 M)	110 μ l
Protease Inhibitor Cocktail*	1 vial*

*Add 250 μ l of DMSO and mix well before use.

III. Mitochondria/Cytosol Fractionation Protocol:

A. General Considerations and Reagent Preparation:

- Read the entire protocol before beginning the procedure.
- After opening the kit, store buffers at 4°C. Store Protease Inhibitor Cocktail and DTT at -20°C.
- Make 1x Cytosolic Extraction Buffer by mixing the 20 ml of 5x buffer with 80 ml ddH₂O.
- Prepare enough Mitochondria Extraction Buffer Mix and Cytosol Extraction Buffer Mix for your experiment: Add 2 μ l Protease Inhibitor Cocktail and 1 μ l DTT to 1 ml of Mitochondria Extraction Buffer and to 1 ml of 1x Cytosol Extraction Buffer, individually, before use.
- Be sure to keep all buffers on ice at all times during the experiment.

B. Cell Fractionation Protocol:

1. Induce apoptosis in cells by desired method. Concurrently incubate a control culture *without* induction.
2. Collect cells (5×10^7) by centrifugation at 600 x g for 5 min. at +4°C.
3. Wash cells with 10 ml of ice-cold PBS. Centrifuge at 600 x g for 5 min. at 4°C. Remove supernatant.
4. Resuspend cells with 1 ml of 1x Cytosol Extraction Buffer Mix containing DTT and Protease Inhibitors (prepared as in Section A).
5. Incubate on ice for 10 min.

6. Homogenize cells in an ice-cold dounce tissue grinder. Perform the task with the grinder on ice. We recommend 30-50 passes with the grinder; however, efficient homogenization may depend on the cell type.

Note: To check the efficiency of homogenization, pipette 2-3 μ l of the homogenized suspension onto a coverslip and observe under a microscope. A shiny ring around the nuclei indicates that cells are still intact. If 70-80% of the nuclei do not have the shiny ring, proceed to step 7. Otherwise, perform 10-20 additional passes using the dounce tissue grinder.

Excessive homogenization should also be avoided, as it can cause damage to the mitochondrial membrane which triggers release of mitochondrial components.

7. Transfer homogenate to a fresh 1.5-ml tube, and centrifuge at 700 x g (~3'000 rpm) for 10 min. at 4°C. Collect the supernatant carefully and discard the pellet.
8. Transfer supernatant to a fresh 1.5-ml tube, and centrifuge at 10'000 x g (~13'000 rpm) for 30 min. at 4°C. Collect supernatant and save the pellet.
9. Collect supernatant from step 8 as **Cytosolic Fraction** (Store at -80°C).
10. If intact mitochondria are desired, resuspend the pellet from step 8 in 0.1 ml 1x PBS (Not provided). These are the intact mitochondria.

If mitochondrial protein lysate is desired, resuspend the pellet from Step 8 in 100 μ l of the Mitochondrial Extraction Buffer Mix containing DTT and protease inhibitors (as prepared in section A), vortex for 10 sec. and save as **Mitochondrial Fraction** (Store at -80°C).

IV. Storage:

- After opening the kit, store buffers at +4°C.
- Store Protease Inhibitor Cocktail and DTT at -20°C.
- Store Cytosolic and Mitochondrial Fraction at -80°C.