ReadiPrep™ Mitochondrial/Cytoplasmic Fractionation Kit

Catalog number: 60005
Unit size: 50 Tests

Component	Storage	Amount (Cat No. 60005)
Component A: Isolation Buffer	Refrigerated (2-8 °C), Minimize light exposure	1 bottle (100 mL)
Component B: Reagent A	Minimize light exposure, Freeze (< -15 °C)	1 vial (1 mL)

OVERVIEW

The ReadiPrep™ Mitochondrial/Cytoplasmic Fractionation Kit offers a convenient method for isolating mitochondria and cytoplasmic fractions from mammalian cells or tissue. The isolated mitochondria are intact and can be positively stained with a mitochondria marker (such as MitoLite™ Green). The isolated mitochondria preserve their biological activities and are compatible with many downstream applications including the study of mitochondrial respiration, mitochondria membrane potential, apoptosis, mtDAN and mtRNA, and mitochondrial protein profiling etc. The kit offers two options for the isolation of mitochondria. One option utilizes a reagent-based method which allows multiple sample preparation at the same time. The second option utilizes Dounce homogenization.

AT A GLANCE

Protocol Summary

1. Wash cells with PBS
2. Add 2 mL of Isolation Buffer
3. Incubate on ice for 10 minutes
4. Homogenize the cells using Homogenizer or add Reagent A.
5. Centrifuge at 7000 g for 10 mins
6. Remove the supernatent (Cytoplasmic Fraction)
7. Resuspend the pellets in Isolation Buffer
8. Centrifuge at 7000 g for 10 minutes
9. Resuspend the pellets in Assay Buffer (Mitochondria Fraction)

Important Note

Thaw all the kit components to room temperature before starting the experiment. Keep the buffer on ice during the experiment.

SAMPLE EXPERIMENTAL PROTOCOL

1. Wash the cells once with cold PBS. For cells in suspension, collect the cells by centrifugation.
2. Add 2 mL of Isolation Buffer (Component A) to cells and incubate on ice for 10 minutes.
3. Two ways cytoplasmic fraction can be extraced. Option A: Add 40 µL of Reagent A (Component B) into the mix, vortex and incubate on ice for 10 minutes. Option B: Homogenize the cells using a Dounce Tissue Grinder by stroking 4-5 times.
4. Centrifuge the sample at 600 g for 10 minutes at 4 °C and collect the supernatent and centrifuge again at 7,000 g for 10 minutes at 4 °C. The supernatent is cytoplasmic fraction.
5. Re-suspend the pellet in 1 mL of Isolation Buffer (Component A) and centrifuge at 7,000 g for 10 minutes at 4 °C.
6. Discard the supernatent, and resuspend the pellet in the appropriate assay buffer based on downstream applications.
7. The protein concentration can be quantified by Amplite™ Fluorimetric Fluorescamine Protein Quantitation Kit (Cat# 11100).

EXAMPLE DATA ANALYSIS AND FIGURES

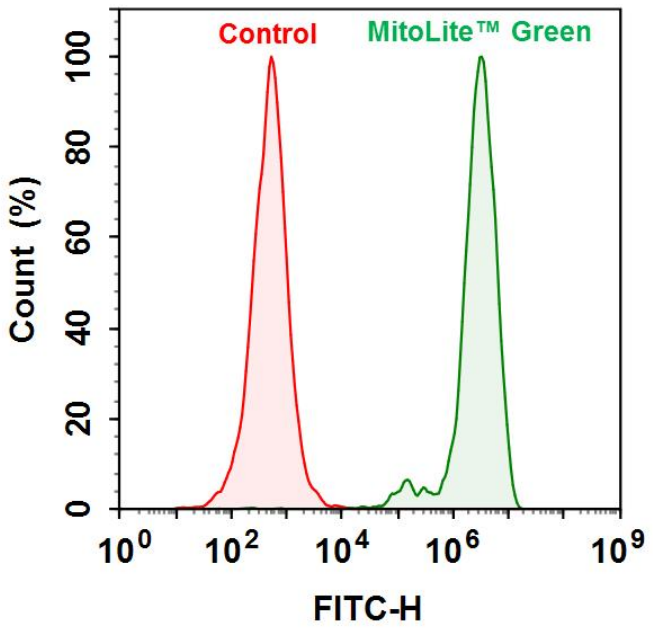

Figure 1. Mitochondria fractions from HeLa cells were collected using ReadiPrep™ Mitochondrial/Cytoplasmic Fractionation Kit. The protein was quantified by Amplite® Fluorimetric Fluorescamine Protein Quantitation Kit (#11100). 100 µg of mitochondria fragment was incubated with or without MitoLite™ Green (#22675) and assayed with a NovoCyte 3000 flow cytometer.

DISCLAIMER

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