Mitochondrial Protein IP Kit

(Catalog #BN00543; 50 assays; Store at -20°C)

I. Introduction:

Mitochondria are the power house of the cells and play an essential role in energy production. Damage to the mitochondria activates signaling pathways that induce apoptosis. Mitochondria also regulate and mediate transport of the metabolites and ions needed for oxidative phosphorylation and maintenance of membrane potential for ATP synthesis. Mitochondrial dysfunction leads to several disorders like cardiac dysfunction, diabetes, aging and neurological disorder, mainly caused by mutations in mitochondrial DNA or in nuclear genes that code for mitochondrial components. Thus, mitochondria have several different functions in the cell.

Assay Genie's ready to use mitochondria Protein IP Buffer is optimized for immunoprecipitation (IP and co-IP) using mitochondria and mitochondrial extracts. The buffer is a gentle formulation, which maintains the stability of mitochondrial complexes. The Mitochondrial Protein IP kit is provided with different choices of detergents like n-Dodecyl-beta-D-maltoside, Triton X-100 and digitonin to achieve different stringency conditions for protein-protein interaction studies. Triton X-100 is the most commonly used detergent especially for membrane protein solubilization. However, in case of fragile complexes digitonin or n-Dodecyl-beta-D-maltoside is the choice of detergents.

II. Application:

- Optimized for compatibility with immunoprecipitation (IP and co-IP) and pull-down using tagged proteins.
- Gentle formulation for maintenance of stable mitochondrial complexes.
- Compatible with SDS PAGE, 2D gel, Blue-Native gel, Mass spectrometry.
- Functional Assays and enzymatic assays.

III. Sample Type:

- Mitochondria, mitochondrial extract or cell lysate.

IV. Kit Contents:

Components	BN00543	Cap Code	Part Number
Mitochondria Protein IP Buffer	50 ml	WM	BN00543-1
Wash Buffer (5X)	50 ml	NM	BN00543-2
Protease Inhibitor Cocktail	Lyophilized	Red	BN00543-3
10% n-Dodecyl-beta-D-maltoside	1 ml	Green	BN00543-4
10% Triton X-100	1 ml	Orange	BN00543-5
10% Digitonin	1 ml	Blue	BN00543-6

V. User Supplied Reagents & Equipments:

- Antibody, protein A/G beads, nutator, SDS-PAGE gel loading buffer, DMSO.

VI. Storage and Handling:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the experiment.

VII. Reagent Preparation and Storage Conditions:

- **Protease Inhibitor Cocktail:** Resuspend protease inhibitor cocktail in 250 µl of DMSO. Store at-20°C. Stable for six months if stored properly.
- **Wash Buffer:** Make 1X Wash Buffer (1:5 dilutions) and add same detergent used for lysis to final conc. of 0.1 %. Store at 4°C.
- **Detergents:** Bring all three detergents to room temperature. Quickly vortex to dissolve any visible precipitation. Store at room temperature.

VIII. Protocol:

A. Sample Preparation and Solubilization: Isolated mitochondria from desired source (e.g. cells, tissue or yeast) are prepared using Assay Genie's Mitochondria/Cytosol Fractionation Kit or Yeast Mitochondria Isolation Kit. Purified mitochondria are solubilized in a non-ionic detergent. Three different detergents are provided in the kit to determine the best IP/Co-IP/Pull down scenario. Detergents solubilization process disrupts the membrane and keeps membrane embedded multi-subunit complexes intact.

1. Take isolated mitochondria or mitochondrial suspension (Yeast ~200 µg; Cell ~1 mg: Whole tissue ~200-300 µg). Add Mitochondria Protein IP Buffer provided with the Kit such that the protein concentration is ~1 mg/ml. Mix well (gentle vortex) and add 1/10 volume of 10% detergent (final concentration 1%). Add 1µl of protease inhibitor cocktail and incubate on ice for 30 minutes.
2. Centrifuge at 12,000 g for 10 minutes at 4°C in a bench top ultracentrifuge and collect the supernatant. Keep the sample on ice until immunoprecipitation is performed.

B. Immunoprecipitation:

1. Add desired amount of polyclonal or monoclonal antibody of interest to the solubilized mitochondrial supernatant. Allow this mixture to mix for at least 3 hours at room temperature or overnight at 4°C on nutator.
2. Add Protein A/G beads ~100 µl (prewashed with PBS) to the mixture and incubate for 1 hour at 4°C on a nutator. Collect the beads by centrifugation for 1 minute at 3,000g on a bench top microfuges. Remove the supernatant from the beads. This represents unbound proteins.

3. Wash the beads to remove any non-specifically bound proteins prior to elution by adding 2 volumes of 1X Wash Buffer containing detergent to the beads. Gently mix for 5 minutes by inverting and collect the beads by centrifugation as performed in Step 2. Remove the Wash Buffer from the beads and discard. Repeat washing step 2-3 times depending on the stringency.
4. Elute the complex by adding 50 µl of SDS-PAGE gel loading buffer. The purified complexes have now been released into the supernatant which should be collected from above the beads. Repeat the elution twice to get the maximum elution of the complex.
5. This sample can be used for further downstream application like SDS-PAGE, 2D gel electrophoresis or Mass spectrometery.

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