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Product Information

Mitochondrial Protein Immunoprecipitation (IP) Kit

Catalog Number **MTP001**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Mitochondria are well known to play an essential role in the energy production process of cells. They are also key in signalling a wide range of cellular function events. In addition to the role of mitchondria in regulation and transport of the metabolites and ions needed for oxidative phosphorylation and maintenance of membrane potential for ATP synthesis, the mitochondira are integrally involved in biogenesis, regulating cell function, and signaling apoptosis in response to stress. 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.

The Mitochondrial Protein Immunoprecipitation (IP) kit contains a ready to use Mitochondria Protein IP Buffer, 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 Immunoprecipitation (IP) kit also provides choices of detergents, *n*-dodecyl-β-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-β-D-maltoside is the detergent of choice.

The Mitochondrial Protein Immunoprecipitation (IP) kit may be used for the following:

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

Components

Mitochondria Protein IP Buffer (Catalog Number MTP001A)	50 ml
Wash Buffer (Catalog Number MTP001B)	50 ml
Protease Inhibitor Cocktail (Catalog Number MTP001C)	1 vl
10% *n*-dodecyl-β-D-maltoside (Catalog Number MTP001D)	1 ml
10% Triton X-100 (Catalog Number MTP001E)	1 ml
10% Digitonin (Catalog Number MTP001F)	1 ml

Reagents and Equipment Required but Not Provided.

- Primary Antibody
- Protein A/G beads
- SDS-PAGE gel loading buffer
- Nutator mixer

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

The components of this kit are supplied ready to use.

Storage/Stability

Store kit at –20 °C, protected from light. Briefly centrifuge small vials prior to opening.

Procedure

Mitochondrial Sample Preparation

Mitochondria can be isolated using literature procedures or with the following kits:

- MITOISO1- Mitochondria Isolation Kit for animal tissue
- MITOISO2- Mitochondria Isolation Kit for cell samples
- MITOISO3- Yeast Mitochondria Isolation Kit

Mitochondrial Sample Solubilization

Mitchondria 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. The detergent solubilization process disrupts the membrane and keeps membrane embedded multisubunit 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 (Catalog Number MTP001A) to give a final protein concentration is ~1 mg/ml.
2. Mix well (gentle vortex) and add 1/10 volume of 10% detergent (final detergent concentration 1%).
3. Add 1 µl of protease inhibitor cocktail and incubate on ice for 30 minutes.
4. Centrifuge at 12,000 × *g* for 10 minutes at 2–8 °C in a bench top ultracentrifuge and collect the supernatant. Keep the sample on ice until immunoprecipitation is performed.

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 2–8 °C on a nutator mixer.
2. Add Protein A/G beads (~100 µl, prewashed with PBS) to the mixture and incubate for 1 hour at 2-8 °C on a nutator mixer. Collect the beads by centrifuging for 1 minute at 3,000 × *g* on a bench top microfuge. Remove the supernatant from the beads. This represents unbound proteins.
3. Wash the beads to remove any non-specifically bound proteins by adding 2 volumes of 1× 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.
4. Remove the Wash Buffer from the beads and discard. Repeat depending on the stringency.
5. 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.
6. This sample can be used for further downstream application like SDS-PAGE, 2D gel electrophoresis, or mass spectrometery.

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