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ab239710 Mitochondrial Protein IP Kit

For immunoprecipitation (IP and co-IP) using mitochondria, mitochondrial extract or cell lysate.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Mitochondrial Protein IP Kit (ab239710) 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.

2. Protocol Summary

Prepare isolated mitochondria from desired source

Add Mitochondria Protein IP Buffer. Mix well and add detergent. Add protease inhibitor cocktail and incubate on ice for 30 min.

Centrifuge at 12,000 x *g* for 10 min at 4°C and collect the supernatant. Keep the sample on ice.

Add antibody of interest to the solubilized mitochondrial supernatant. Allow to mix for at least 3 h at RT or overnight at 4°C on nutator.

Add Protein A/G beads and incubate for 1 h at 4°C on a nutator. Collect the beads by centrifugation for 1 min at 3,000 x *g*. Remove the supernatant. This represents unbound proteins.

Wash the beads. Elute by adding 2 volumes of 1X Wash Buffer containing detergent. Gently mix for 5 min by inverting and collect the beads by centrifugation. Remove Wash Buffer from the beads and discard. Repeat washing step 2-3 times depending on the stringency.

Elute the complex by adding SDS-PAGE gel loading buffer. Collect the supernatant from above the beads. Repeat the elution twice to get the maximum elution of the complex.

3. General guidelines, precautions, and troubleshooting

- Please observe safe laboratory practice and consult the safety datasheet.
- For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
- For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

- Store kit at -20°C in the dark immediately upon receipt and check below in Section 6 for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
- Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage condition
Mitochondria Protein IP Buffer	50 mL	-20°C
Wash Buffer (5X)	50 mL	-20°C
Protease Inhibitor Cocktail	1 vial	-20°C
10% n-Dodecyl-beta-D-maltoside	1 mL	-20°C
10% Triton X-100	1 mL	-20°C
10% Digitonin	1 mL	-20°C

5. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Antibody.
- Protein A/G beads.
- Nutator.
- SDS-PAGE gel loading buffer.
- DMSO.

6. Reagent Preparation

- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- Prepare only as much reagent as is needed on the day of the experiment.

6.1 Mitochondria Protein IP Buffer:

Store at -20°C. Bring to room temperature (RT) before use.

6.2 Wash Buffer (5X):

Make 1X Wash Buffer (1:5 dilutions) and add same detergent used for lysis to final concentration of 0.1 %. Store at 4°C.

6.3 Protease Inhibitor Cocktail:

Resuspend protease inhibitor cocktail in 250 µL of DMSO. Store at-20°C. Stable for six months if stored properly.

6.4 10% n-Dodecyl-beta-D-maltoside:

Bring to room temperature. Quickly vortex to dissolve any visible precipitation. Store at room temperature.

6.5 10% Triton X-100:

Bring to room temperature. Quickly vortex to dissolve any visible precipitation. Store at room temperature.

6.6 10% Digitonin:

Bring to room temperature. Quickly vortex to dissolve any visible precipitation. Store at room temperature.

7. Sample Preparation

Isolated mitochondria from desired source (e.g. cells, tissue or yeast) are prepared using Mitochondria/Cytosol Fractionation Kit (ab65320) or a 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.

7.1 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.

7.2 Mix well (gentle vortex) and add 1/10 volume of 10% detergent (final concentration 1%).

7.3 Add 1µL of protease inhibitor cocktail and incubate on ice for 30 min.

7.4 Centrifuge at 12,000 x *g* for 10 min at 4°C in a bench top ultracentrifuge and collect the supernatant.

7.5 Keep the sample on ice until immunoprecipitation is performed.

8. Assay Procedure

8.1 Add desired amount of polyclonal or monoclonal antibody of interest to the solubilized mitochondrial supernatant. Allow this mixture to mix for at least 3 h at room temperature or overnight at 4°C on nutator.

8.2 Add Protein A/G beads ~100 µL (prewashed with PBS) to the mixture and incubate for 1 h at 4°C on a nutator. Collect the beads by centrifugation for 1 min at 3,000 x *g* on a bench top microfuge. Remove the supernatant from the beads. This represents unbound proteins.

8.3 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 min by inverting and collect the beads by centrifugation as performed in Step 8.2. Remove the Wash Buffer from the beads and discard. Repeat washing step 2-3 times depending on the stringency.

8.4 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.

8.5 This sample can be used for further downstream application like SDS-PAGE, 2D gel electrophoresis or Mass spectrometry.

10. Notes

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