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ab109711
Complex I Immunocapture Kit

View Complex I Immunocapture Kit datasheet:

www.abcam.com/ab109711

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For the isolation of Complex I from small amounts of Human, Rat, Mouse and Bovine tissue

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

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1. Overview

ab109711 allows for isolation of Complex I from small amounts of tissue. This facilitates subsequent analysis of assembly state and activity. Thus the enzyme retains NADH-ferricyanide, NADH-CoQ1 and NADH hexaaminoruthenium reductase activities after isolation, and with added lipids it also shows significant rotenone sensitivity.

Finally, the extent of post translational modifications including oxidative damage can be readily analyzed by proteomic approaches or antibody detection of these modifications. Uses for ab109711 include but are not limited to examining alterations of Complex I subunits in inherited mitochondrial diseases, Parkinson's disease, Alzheimer's disease, ALS, schizophrenia, and aging.

Note: The immunocapture protocol for this kit requires Abcam detergent (Lauryl Maltoside, ab109857).

2. Quick Assay Procedure

Δ Note: this procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

Resuspend mitochondria to 5.5 mg/ml in PBS. Add 1X protease inhibitors

Take 0.18 ml (1 mg) and add 20 µl 10% LM incubate on ice 30 minutes

Centrifuge 25,000g 4°C for 10 minutes

Incubate supernatant with ~10 µl solid beads 3 hours at room temperature OR overnight 4°C

Beads collected by gentle centrifugation (2 minutes, 200g) and washed with Wash buffer (1X PBS/0.05% LM), repeat three times.

Complex eluted from the resin by addition of Glycine, SDS, or Urea Elution Buffer

Protein concentration determined

3. Materials Supplied and Storage

Store kit at 4°C immediately on receipt. Kit can be stored for 1 year from receipt.

Avoid repeated freeze-thaws of reagents.

4. Materials Required, Not Supplied

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

- n-dodecyl-β-D-maltopyranoside (Lauryl maltoside (LM), ab10985)
- Phosphate buffered saline, 1X PBS (1.4 mM KH2PO4, 8 mM Na2HPO4, 140 mM NaCl, 2.7 mM KCl, pH 7.3)
- Wash buffer: 1XPBS, 0.05% LM
- Elution buffer
    - Glycine (0.2 M Glycine. HCl pH 2.5)
    - SDS (1% sodium dodecyl sulfate)
    - Urea elution buffer (4 M Urea. HCl pH 7.5)
- Neutrolization buffer: 1M Tris-HCl pH 7.8
- Protease inhibitor cocktail (1 M phenylmethanesulfonyl fluoride (PMSF) in acetone, 1 mg/ml leupeptin, 1 mg/ml pepstatin)
- Double distilled water
- Laboratory benchtop microfuge
- Protein electrophoresis equipment
- Tube rotation equipment
- pH meter, weighing balance and other standard lab equipment

5. 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.

6. Sample Preparation

General sample information:

When performing an immunocapture procedure, it is always recommended to isolate mitochondria from cells before immunoprecipitation. It is possible to isolate complexes from whole tissue or cell extract, although this may result in a weaker signal and/or additional bands resulting from non-specific cross-reactivity.

Abcam immunocapture antibodies have been optimized to immunoprecipitate the OXPHOS and PDH complexes from a wide range species. A minimal amount of starting mitochondria/cells is critical. Suggested minimum amounts of mitochondria as starting material are presented in Table 1.

Sample	Minimal Starting Amount	Recommend Starting Amount
Heart mitochondria	100 µg	1-5 mg
Muscle mitochondria	200 µg	2-5 mg
Brain mitochondria	300 µg	5 mg
Cultured cell mitochondria	1 mg	5 mg
Cultured cell extract	6 mg	15 mg

Table 1. Suggested minimal starting amounts.

The total amount of OXPHOS complexes in mitochondrial samples varies greatly between species and tissue types. It is highly recommended that during the experimental planning steps an estimation is made of the total amount of the complex in the user's sample. Table 2 suggests detection strategies based upon anticipated yield of immunocaptured product.

Yield of Complex	Detection Strategy
1 µg+	Gel staining with Coomassie
10 ng+	Gel staining with silver/sypro ruby
1 ng+	Western blotting
Any	Mass spectrometry

Table 2. Suggested detection methods by yield of product

7. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.
- Assay all standards, controls and samples in duplicate.

7.1 Sample Solubilization

The sample should be solubilized in a non-ionic detergent. It has been determined that at a protein concentration of 5 mg/ml mitochondria are completely solubilized by 20 mM n-dodecyl-β-D-maltopyranoside (1% w/v lauryl maltoside). The key to this solubilization process is that the membranes are disrupted while the previously membrane embedded multisubunit OXPHOS complexes remain intact, a step necessary for the antibody based purification procedure described below.*

1. To a mitochondrial membrane suspension at 5.5 mg/ml protein in PBS add 1/10 volume of 10% lauryl maltoside (final concentration of 1%).
2. Mix well and incubate on ice for 30 minutes.
3. Centrifuge at 25,000 g for 10 minutes. At a minimum, a benchtop microfuge on maximum speed, usually around 16,000 g should suffice.
4. Collect the supernatant and discard the pellet.
   Note: Samples rich in mitochondria such as heart mitochondria, the cytochromes in Complexes II and III should give this supernatant a brown coloration.
5. Add a protease inhibitor cocktail to the mixture and keep the sample on ice until immunoprecipitation is performed.

Note: One important exception to this solubilization method is the pyruvate dehydrogenase enzyme. In order to isolate PDH at a protein concentration of 5 mg/ml mitochondria the required detergent concentration is only 10 mM (0.05%) lauryl maltoside.

The PDH enzyme should also be centrifuged at lower speed, at a max centrifugal force of 16,000 g is maximum for the PDH complex.

7.2 Immunoprecipitation:

Abcams' immunocapture beads are agarose beads irreversibly cross-linked to highly specific monoclonal antibodies capable of binding and retaining specific OXPHOS complexes or the PDH complex. The loaded beads are completely saturated with antibody to ensure the maximum amount of antibody per bead volume and hence the maximum amount of immunoprecipitated product.

The smallest working amount of beads is 5 µl of solid beads in a small microtube (e.g. a 500 µl tube). It is not practical to use a volume smaller than this for immunocapture procedure, therefore it is recommended that when wishing to use less beads researchers dilute the concentrated Abcam's beads with unloaded agarose beads. The yield from such diluted beads will be less.

As an alternative, the immunocapture antibody (ab109798), unbound agarose beads *(ab174816).* and the cross-linking antibody to beads protocol are also available from Abcam.

7.2.1 Add the desired amount of antibody loaded agarose beads (i.e. at least 5 µl of the solid beads) to the appropriate amount of solubilized mitochondrial supernatant

7.2.2 Allow this mixture to mix for at least 3 hours at room temperature or overnight at 4°C. Mixing should be done on a nutator or a rotator.

7.3 Elution:

After the mixing step is complete, collect the beads by centrifugation for 2 minute at 200g

7.3.1 Carefully remove the supernatant from above the beads and discard.

7.3.2 Wash the beads three times to remove any non-specifically bound proteins prior to elution by adding 100 volumes of Wash buffer (1XPBS/0.05% LM) to the beads.

7.3.3 Gently mix for 5 minutes in each wash. Collect the beads by gentle centrifugation for 2 min at 200g.

7.3.4 Remove the wash buffer from above the beads and discard. Repeat the washing process two more times.

7.3.5 In the final step, all wash buffer is removed from above the beads. The complex is now ready for elution.

Three options are available for elution of the bound complex from the beads. The chosen elution procedure should be performed on the beads three times. This ensures complete removal of target from the beads. The three samples should be collected and analyzed, samples containing the most protein should then be pooled.

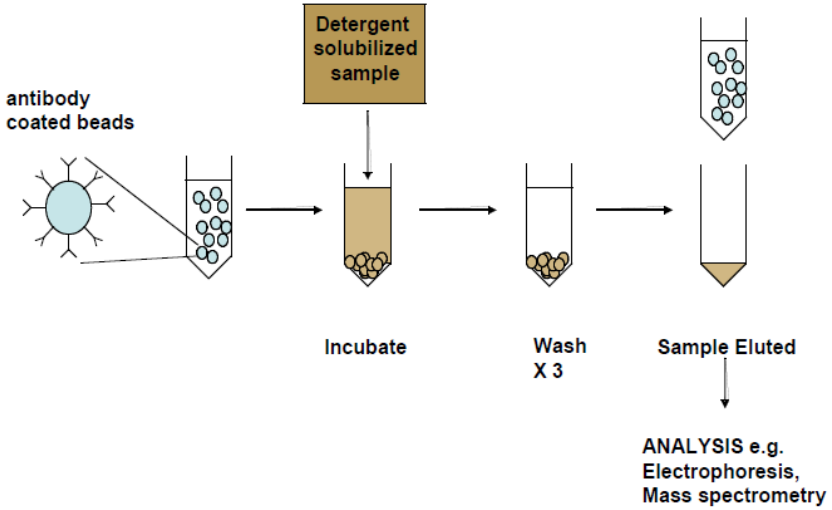
7.3.6 Resuspend the beads in 2-5 volumes of elution buffer. Gently mix for 10 minutes. Collect the supernatant by centrifuging for 5 min at 10,000g

Possible buffers are:

- Glycine buffer elution – the complex is eluted from the beads by acidification. This method is advantageous because the beads can be reusable after removal of glycine elution buffer by washing the beads as described above. However, the eluted sample has an acidic pH which needs to be neutralized before analysis (see 11.2).

- SDS buffer elution – the complex is eluted from the beads by the denaturant SDS. This method is advantageous because the extraction method is highly efficient and therefore the sample is more concentrated. However, the antibody on the beads is no longer viable due to denaturing by the SDS. Beads should be discarded after use.

- Urea buffer elution - the complex is eluted from the beads by the chaotrope urea. This is advantageous because the sample can be processed further with proteolytic enzymes for mass spectrometry. The sample eluted with urea should be diluted at least four fold to reduce the urea concentration to levels permitting proteolysis by trypsin.


Detergent solubilized sample
antibody coated beads
Incubate
Wash X 3
Sample Eluted
ANALYSIS e.g. Electrophoresis, Mass spectrometry

8. Sample Analysis

Samples eluted by any of the three methods can now be resolved by electrophoresis. Resolved proteins should be detected by the method chosen in Table II.

No antibody should be present in the sample once eluted from the beads; therefore prior to loading, the sample can be reduced by a reducing agent such as 50 mM DTT or 1% β-mercaptoethanol. Another optional step is heating of the sample which can be done at 95°C for 5 minutes or at 37°C for 30 minutes prior to loading onto the gel. These steps may increase the resolution of protein bands and also reduce the complexity of the sample by breaking any disulfide bonded proteins.

Note: *Heated samples need to cool down to room temperature before loading.*

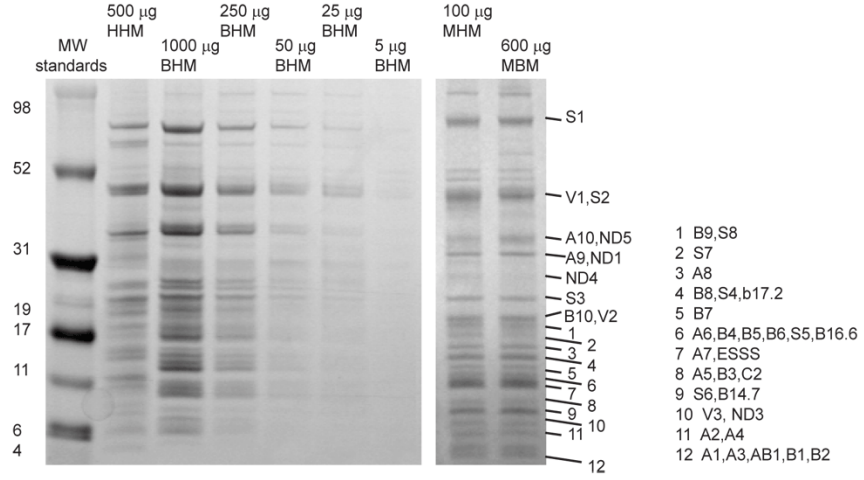


Figure 1. Complex I immunoprecipitation using antibody ab109798 /MS101c cross-linked to protein G-agarose beads as product ab109711

9. Species Reactivity

Species Reactivity: Human, rat, mouse, bovine

10. FAQs / Troubleshooting

General troubleshooting points are found at www.abcam.com/assaykitguidelines,

Problem	Solution
Antibody contamination	When eluting, pipette only the liquid sample from above the beads. This sample can be spun again and aspirated to ensure that it is free of beads If there is anysuspicion of antibody contamination, avoid using reducing agent in sample. This would maintain antibody at 200 kDa during electrophoresis
Sample turns yellow with loading buffer addition	Neutralize the acidity with Tris Base
Large CV	Check pipettes
Weak or no signal	Increase the bead amount Increase the amount of sample Increase sample/bead incubation time use enrich mitochondria as sample
Non-specific bands	Isolate mitochondria to higher purity Add a reducing agent to the eluted sample e.g. DTT Heat the eluted sample at 95°C for 5 min before loading

11. Optimization Steps and General Tips

11.1 Using agarose bead slurries: Since the beads are a solid material they cannot be pipetted directly. Instead they are provided in a much larger volume of liquid (usually 10 volumes of HBS). Pipetting this solution up and down mixes the beads into a slurry allowing their transfer to the experimental tube. This is the only step in this protocol the beads are pipetted directly. Minimize mixing beads by pipetting through out the procedure since bead losses can occur on the inside of the pipette tip. Also, avoid vigorous mixing during the elution step when the liquid volume is low. Beads might become deposited around the inside tube unexposed to elution buffer leading to losses. Instead gently tap the tube to agitate the beads within the small volume of sample elution buffer.

11.2 Samples eluted by Glycine buffer: Sample eluted by glycine buffer, pH 2.5, needs to be neutralized with equal volume of 1M Tris-HCl pH7.8, before adding SDS-PAGE loading buffer. Skipping neutralizing step would cause calor change from blue to yellow as the bromophenol blue in the SDS-PAGE loading buffer acts as a pH indicator.

11.3 Sample concentration: When analyzing immunocaptured products by SDS-PAGE, it is recommended to load as much sample as possible on the gel.

11.4 Blot development: When analyzing immunocaptured products by Western blotting choose an appropriate method for blot development. The alkaline phosphatase (NBT/BCIP) and horseradish peroxidase (ECL) methods are recommended.

12. Notes

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