



ab133988 –

PDH Rodent

Immunocapture Kit

Instructions for Use

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

Table of Contents

1. Introduction	3
2. Assay Summary	4
3. Kit Contents	5
4. Storage and Handling	6
5. Additional Materials Required	6
6. Preparation of Reagents	7
7. Sample Preparation	8
8. Assay procedure	10
9. Antigen Elution	11
10. Sample Analysis	13
11. Specificity	15
12. Optimization Steps and General Tips	15
13. Troubleshooting	16

1. Introduction

Abcam PDH Rodent Immunocapture Kit is suitable to immunocapture PDH complex from rodent tissue homogenates and whole cells lysates. Traditional immunoprecipitation methods typically result in co-elution of the antibody heavy and light chains that may co-migrate with relevant bands, masking important results. The Abcam PDH Rodent Immunocapture Kit resolves this issue by immobilizing the PDH capture antibody onto protein G-agarose beads. The kit includes optimized buffers and reagents for sample preparation, PDH binding and recovery, which shorten the protocol and minimize handling and mixing.

Background: The pyruvate dehydrogenase complex performs the decarboxylation of pyruvate into acetyl CoA, a critical function in metabolism, linking glycolysis and oxidative phosphorylation in mitochondria. The enzyme is composed of multiple copies of three enzymes: pyruvate dehydrogenase (E1), dihydrolipoamide transacetylase (E2) and dihydrolipoamide dehydrogenase (E3). The E1 enzyme is a tetramer of two alpha (PDHA1) and two beta (PDHB) subunits.

2. Assay Summary

Resuspend rodent samples to 5.5 mg/mL in PBS



Solubilize rodent samples by adding proper volume of 10X Extraction Buffer, mix thoroughly, incubate on ice for 20 minutes



Centrifuge at 5,000 g for 10 minutes at 4°C, collect supernatant.



Incubate supernatant with diluted beads for 3 hours at 4°C



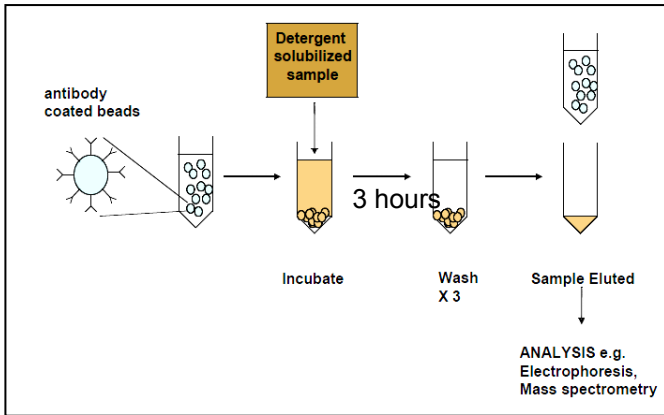
Collect beads by gentle centrifugation (2min x300 g) and wash the beads three times in wash buffer. Collect beads by gentle centrifugation (2 min x 300g) after each washing step.



Elute PDH Complex from the beads by adding proper volume of Glycine elution buffer; or SDS elution buffer



Analyze the eluted sample by protein electrophoresis or store the purified PDH at -80°C



3. Kit Contents

Item	Quantity
10 X Wash Buffer	10 mL
10 X PBS	10 mL
10 X Extraction Buffer	1 mL
Immunocapture Antibody Coupled Agarose Beads	100 μ L
SDS Elution Buffer	1 ml
Glycine Elution buffer	1 ml
Neutralization buffer	1 ml

4. Storage and Handling

Store all components at 4°C. This kit is stable for 6 months from receipt.

5. Additional Materials Required

- Method for determining protein concentration (BCA assay recommended)
- Nanopure water
- Single-channel pipettes
- Protease inhibitor cocktail (e.g. ab65621 from Abcam)
- Laboratory benchtop microcentrifuge
- Protein electrophoresis equipment
- Tube rotation equipment
- pH meter, weigh balance and other standard lab equipment

6. Preparation of Reagents

- 6.1 Equilibrate all reagents and samples to room temperature (18 - 25°C) prior to use.
- 6.2 Prepare 1X Wash Buffer by adding 10 mL 10X Buffer to 90 mL nanopure water.
- 6.3 Prepare 1X PBS by adding 10 mL 10X PBS to 90 mL nanopure water.
- 6.4 If preparing lysates from cell pellets prepare 1X Extraction Buffer from 10X Extraction Buffer by adding 1 Volume of 10X Extraction Buffer to 9 volumes of nanopure water, e.g. add 4.5 mL nanopure water to 0.5 mL 10X Extraction Buffer
If preparing lysates from cell or tissue suspensions the 10X Extraction buffer is used. Store all the diluted reagents at 4°C after use.

7. Sample Preparation

Note: Extraction buffer can be supplemented with protease inhibitor cocktail (e.g. ab65621) prior to use. Supplements should be used according to manufacturer's instructions.

7.1 Tissue lysates

- 7.1.1. Tissue lysates are typically prepared by homogenization of tissue that is first minced and thoroughly rinsed in PBS to remove blood (dounce homogenizer recommended).
- 7.1.2. The protein concentration may be quantified from a small aliquot of the homogenate using a protein assay (e.g. BCA assay).
- 7.1.3. Suspend the homogenate to 5.5 mg/mL in PBS.
- 7.1.4. Solubilize the homogenate by adding 1 volume of 10X Extraction Buffer to 9 volumes of the homogenate, e.g. 20 μ L 10X Extraction Buffer to 180 μ L homogenate (= 1 mg protein), thoroughly mixed.
- 7.1.5. Incubate the mixture on ice for 20 minutes. Centrifuge 5,000 x g for 10 minutes at 4°C. Transfer the supernatant into a clean microtube and discard the pellet. Assay samples immediately or aliquot and store at -80°C.

7.2 Cell lysates

7.2.1. Collect non-adherent cells by centrifugation or scrape to collect adherent cells from the culture flask. If the adherent cells are detaching, collect both floating cells and remaining adherent cells. Typical centrifugation conditions for cells are 500 x g for 10 min at 4°C.

7.2.2. Rinse cells twice with PBS.

7.2.3. Solubilize cell pellet at 2×10^7 /mL in 1X Extraction Buffer or simply add 9 volumes of 1X Extraction Buffer to one volume of cell pellet, thoroughly mixed.

7.2.4. Incubate the mixture on ice for 20 minutes. Centrifuge 5,000 x g for 10 minutes at 4°C. Transfer the supernatants into clean microtubes and discard the pellets. Assay samples immediately or aliquot and store at -80°C for 6 months. The sample protein concentration in the extract may be quantified using a protein assay.

A minimal amount of starting materials (tissues/ cells) is critical. Suggested minimum amounts of starting material are presented in table 1.

Material	Minimal starting amount
Muscle/heart tissue homogenate	0.5 mg - 1 mg
Other tissue homogenates	0.75 mg – 1 mg
Whole cell homogenates	1 mg – 2 mg
Mitochondria extract	0.25 mg – 1 mg

Table1. Suggested minimal starting amount.

8. Assay procedure

Note: It is strongly recommended to use a positive control sample along with the tested samples. The sample should be solubilized following the description in sample preparation section. The smallest working amount of beads is around 5 μ l of solid beads in a microtube (e.g. 1.5 ml). It is not practical to use a volume smaller than this for immunocapture procedure.

- 8.1 Prepare bead slurry by adding the desired amount of antibody loaded beads (i.e. at least 5 μ L of the solid beads) to 1 mL 1X PBS. Add the appropriate amount of solubilized supernatant (see table 1 for reference) to the beads slurry.
- 8.2 Allow this solution to mix for around 3 hours at 4°C using a tube rotator.
- 8.3 After the mixing step is complete, collect the beads by centrifugation for 2 minutes at 300 x g.
- 8.4 Carefully remove the supernatant from above the beads.

- 8.5 Fill the microtube containing beads with 1X Washing Buffer. Gently tap the microtube to ensure the full suspension of the beads.
- 8.6 Collect the beads by centrifugation for 2 minutes at 300 x g.
- 8.7 Repeat steps 8.5 and 8.6 twice to obtain a total of three washes.
- 8.8 Completely remove the supernatant from above the beads. The PDH complex captured on the beads is now ready for elution.

9. Antigen Elution

Note: Abcam PDH immunocapture kit for rodents offers two different elution buffers: (1) Glycine elution buffer for reusable beads and, (2) SDS elution buffer for direct protein electrophoresis.

9.1 Glycine buffer elution:

- 9.1.1. Add 30 μ L Glycine Elution Buffer to each 10 μ L washed beads. Incubate the mixture for 10 minutes with frequent agitation.
- 9.1.2. Collect the beads by centrifugation for 2 minutes at 300 x g.
- 9.1.3. Collect the supernatant from above the beads in a separate microtube. This supernatant contains purified PDH complex.

- 9.1.4. Repeat this process once with the same volume of fresh Glycine Elution Buffer to ensure the maximal elution of the captured PDH complex. Pool the supernatants together.
- 9.1.5. Neutralize the collected supernatant 1:1 with using Neutralization Buffer. Continue to further analysis or store the purified PDH complex at -80°C .

9.2 SDS buffer elution:

- 9.2.1. Add 40 μL SDS Elution Buffer to each 10 μL washed beads. Gently tap the microtube to mix it thoroughly.
- 9.2.2. Collect the beads by centrifuge at 12,000 x g for 5 minute.
- 9.2.3. Collect the supernatant from above the beads in a separate microtube and discard the beads. This supernatant contains denatured purified PDH complex.
- 9.2.4. Continue to further protein electrophoresis by adding proper amount of concentrated Laemmli sample buffer or store the purified PDH complex at -80°C .

10. Sample Analysis

Samples eluted by any of the elution methods can now be resolved by electrophoresis. Resolved proteins should be detected by the method chosen in Table 2.

Yield of PDH 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.

The antibody is cross-linked to the beads therefore no antibody should be present in the sample once eluted from the beads. However, the use of reducing agent such as DTT or β -mercaptoethanol is not recommended.

Some samples may aggregate on boiling; therefore, 95°C heating or boiling of the sample prior to loading is **NOT** recommended. Gentle heating at 37°C for 5-10 minutes may be applied to increase the resolution.

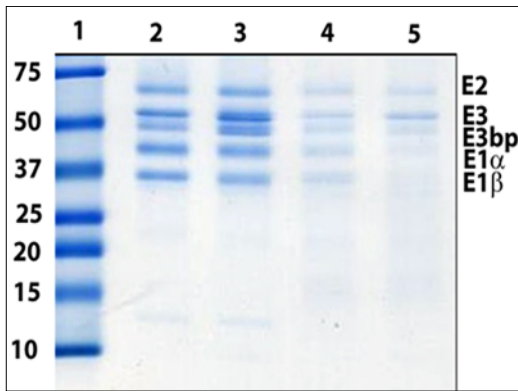


Figure 1. PDH immunoprecipitation from rodent samples using antibody ab133988.

For all lanes: 10 μ L antibody coated beads was mixed with 1 mg rodent sample and incubated for 3 hours. Captured PDH complex was eluted with SDS Elution Buffer and half volume of the eluted PDH complex was loaded on the gel. Gel was stained with Colloidal Coomassie Blue.

Lane 1: Ladder

Lane 2: PDH complex of RHH (rat heart homogenate)

Lane 3: PDH complex of MHH (mouse heart homogenate)

Lane 4: PDH complex of 1 mg H4IIE (rat) whole cell lysate

Lane 5: PDH complex of 1 mg 3T3 (mouse) whole cell lysate

11. Specificity

Species Reactivity: Rat and mouse tissue and whole cell lysates.

12. Optimization Steps and General Tips

Using PDH antibody coated agarose bead slurry: Since the beads are a solid material they cannot be pipetted directly. Instead they are provided in a much larger volume of liquid (e.g. 50 μL of solid beads in 500 μL storage solution). Pipetting this solution up and down mixes the beads into a slurry allowing transfer to the experimental tube. Also, avoid vigorous mixing during the elution step when the liquid volume is low. The insufficient exposure of the antigen bound beads to the elution buffer will reduce the yield of final product. Instead, gently tap the tube to agitate the beads within the small volume of sample elution buffer.

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.

13. Troubleshooting

Problem	Solution
Antibody contamination	When eluting, pipette only the liquid part from above the beads. This supernatant can be spun again and recollected to ensure that it is free of beads.
	Do not include reducing agent in sample during electrophoresis, this should maintain any co-eluted antibody at 150 kDa during electrophoresis.
Sample turns yellow with loading buffer addition	Add neutralization buffer following the protocol.
Weak or no signal	Increase the bead amount.
	Increase sample/bead ratio.
	Increase the incubation time.
Non-specific bands	Supplement protease inhibitor when solubilizing samples.
	Always incubation sample/beads mixture at 4°C.
	Prior to elution, wash the antigen coated beads thoroughly for a total of three times.

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