WSE-7424

EzProteoLysis Native operating instructions

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1. Precautions for safe use of this product

To use this product safely, please read this instruction manual carefully first. Please refrain from operating the product until you fully understand the contents of this instruction manual. This instruction manual describes only how to use this product for the specified purpose. Please refrain from using the product for purposes or in ways not described in this instruction manual. If you use the product for purposes or in ways not described in this instruction manual, you are solely responsible for all necessary safety measures and unforeseen circumstances. Also, please carefully read and understand the instruction manuals of any devices you will be using at the same time.

2. Purpose of use

This kit lyses plant and animal tissues, cultured cells, and other materials to extract native proteins (including membrane proteins). The included protease inhibitors and phosphatase inhibitors prevent protein degradation and dephosphorylation. The extracted protein solution can be used as a sample for Native PAGE, for enzyme activity measurements, electrophoresis, immunoprecipitation, ELISA, and other biochemical and immunological analyses. Because this product does not contain amino or phosphate groups, it can also be used directly in protein labeling experiments.

3. Product configuration

Name	Volume	Qty	Storage
EzProteoLysis Native	30 mL	1	2-10℃
Protease Inhibitor	0.3 mL	1	-20℃ or below
Phosphatase Inhibitor	0.3 mL	1	-20℃ or below

4. Composition

Name	Main component
EzProteoLysis Native	Glycerol, Detergents
Protease Inhibitor	100×Concentration, Aprotinin、Pepstatin, Leupeptin, DMSO
Phosphatase Inhibitor	100x concentration, sodium fluoride, sodium vanadate, sodium glycerophosphate

This Product does not contain any poisonous or deleterious substances under the Poisonous and Deleterious Substances Control Law, or any substances subject to notification that exceed the exemption amounts stipulated under the Industrial Safety and Health Law or the PRTR Law. For details, please download the SDS for this product from the ATTO website (https://www.atto.co.jp/).

5. Storage

- **EzProteoLysis Native** should be stored refrigerated (2-10℃). It can also be frozen (-20℃ or below). If unopened, it will be stable until the expiration date.
- Protease inhibitors and phosphatase inhibitors should be stored frozen (-20℃ or below). Unopened products are stable until their expiration date.
- This product will be shipped refrigerated or frozen and should be stored at the appropriate temperature immediately upon arrival.

6. Disposal method

- Dispose of each reagent in accordance with the disposal method of your institution.

7.What you need other than this product

- Ice-cold PBS buffer
- Microcentrifuge tubes
- Ice bath/coolable block incubator
- Refrigerated centrifuge

8. Precautions for use

- This product will be delivered refrigerated or frozen. Upon arrival, open the package immediately and store at the appropriate temperature for each reagent.
- All reagents should be kept on ice before starting the experiment, and all experimental procedures should be carried out on ice or in a cooled block incubator.
- Since the **Protease inhibitor** contains DMSO, it may freeze at low temperatures. Please thaw it completely at room temperature before use.
- If necessary, increase or decrease the amount of **protease inhibitor** added, or add other inhibitors such as AEBSF or Bestatin.

9. How to use

I. Preparation of EzProteoLysis Native

1. Mix **Protease inhibitor** and **Phosphatase inhibitor** as needed according to the table below.

	Volume	Protease Inhibitor (Blue)	Phosphatase Inhibitor (Brown)
EzProteoLysis Native	1 mL	10µL	10µL

2. After mixing, keep **EzProteoLysis Native** on ice or in a block incubator below 4°C until use.

*The volume of **EzProteoLysis Native** required for cell solubilization is approximately 1 mL for 5 x 10^6 to 2 x 10^7 cells.

II. Solubilization of cultured cells

A. For Adherent Cells

1. Add an appropriate amount of ice-cold PBS to the culture dish in which the cells are cultured and wash the cells at least twice.
2. Add the **EzProteoLysis Native** prepared in **I. Preparation of EzProteoLysis Native** to the PBS-washed cell culture dish.

   *The volume of **EzProteoLysis Native** required for cell solubilization is approximately 1 mL for 5 x 10^6 - 2 x 10^7 cells.
3. Incubate on ice or in a block incubator at 4°C or below for 15 minutes.
4. Scrape the cells from the culture dish with a scraper and collect them in a microcentrifuge tube.
5. Centrifuge at 14,000 x g for 5 - 15 minutes.
6. Collect the supernatant in a new microcentrifuge tube. Keep the solubilized protein solution on ice or at 4°C or below until use. Store at -80°C, avoiding freeze-thawing, and use as soon as possible.

B. For non-adherent cells or cells recovered by trypsinization

1. Prepare a cell suspension recovered by trypsinization or other methods.
2. Centrifuge the cell suspension at 200-500 x g for 3-5 minutes.
3. Carefully discard the supernatant and add an appropriate amount of ice-cold PBS to the cells (precipitates) and suspend them well.
4. Centrifuge the cell suspension at 200-500 x g for 3-5 minutes.
5. Carefully discard the supernatant and suspend the cells (precipitates) in an appropriate amount of ice-cold PBS.
6. Centrifuge the cell suspension at 200-500 x g for 3-5 minutes.
7. Carefully discard the supernatant and add **EzProteoLysis Native** (prepared in **I. Preparation of EzProteoLysis Native**) to the cells (precipitates) and suspend by pipetting.

   *The volume of **EzProteoLysis Native** required for cell solubilization is approximately 1 mL for 5 x 10^6 - 2 x 10^7 cells.
8. Incubate on ice or in a block incubator at 4°C or below for 15 minutes.

   *During incubation, mix by inverting 2-3 times every 2-3 minutes.
9. Centrifuge the cell lysate at 14,000 x g for 5-15 minutes.
10. Collect the supernatant in a new microcentrifuge tube. Keep the solubilized protein solution on ice or at 4°C or below until use. Store at -80°C, avoiding freeze-thawing, and use as soon as possible.

    *If viscosity increases due to DNA elution, treat with sonication or DNase I (100-500 U/mL).

    *Centrifugation at 200-500 x g during cell recovery corresponds to 1,500-2,000 rpm in a refrigerated microcentrifuge with a rotation radius of approximately 8 cm.

    *Centrifugation at 14,000 x g corresponds to 13,000 rpm in a small refrigerated centrifuge with a rotation radius of approximately 8 cm.

III. Chloroplast Solubilization

1. Measure the concentration of isolated chloroplasts. For information on measuring chloroplast concentration, please refer to the following literature.

   *Reference: R.J.Porra et al., "Chlorophylls a and b extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy," Biochim. Biophys. Acta (1989), Vol. 975, 384-394.
2. Add **EzProteoLysis Native** (prepared in **I. Preparation of EzProteoLysis Native**) to the chloroplasts and pipette gently, avoiding foaming.

   *Approximately 1 mL of **EzProteoLysis Native** is required to solubilize approximately 1 mg of chloroplasts.

   *If the chloroplast concentration is too high, incomplete lysis will occur, while if it is too low, over-lysis will occur. Please note that this may result in poor extraction of supercomplexes, OXPHOS, and other complexes.
3. Incubate on ice or in a block incubator at 4°C or below for 15 minutes.

   *During incubation, mix by inverting 2-3 times every 2-3 minutes.

4. Centrifuge the lysate at 14,000 x g for 5-15 minutes.
5. Collect the supernatant in a new microcentrifuge tube. Keep the solubilized protein solution on ice or at 4°C or below until use. Store at -80°C, avoiding freeze-thaw cycles, and use as soon as possible.

IV. Tissue Solubilization

1. Wash approximately 100 mg of tissue by adding an appropriate amount of ice-cold PBS.
2. Add the **EzProteoLysis Native** prepared in **I. Preparation of EzProteoLysis Native** to the washed tissue.

   *Approximately 1 mL of **EzProteoLysis Native** is required to solubilize 100 mg of tissue.*
3. Finely cut the tissue with dissection scissors and homogenize it using a tube homogenizer.
4. Incubate on ice or in a block incubator at 4°C or below for 15 minutes.

   During incubation, mix by inverting the tube 2-3 times every 2-3 minutes.
5. Centrifuge the lysate at 14,000 x g for 5-15 minutes.
6. Collect the supernatant in a new microcentrifuge tube. Store the solubilized protein solution on ice or at 4°C or below until use. Store at -80°C, avoiding freezing and thawing, and use as soon as possible.

10. reference

Sample Preparation for Native-PAGE

1. Add 1/10 volume of **EzApply Native** (**WSE-7011**) to the protein solution or add approximately 10% volume of sugar or glycerin to increase density and mix thoroughly.
2. Centrifuge at 2000-6000 x g for 5 seconds and spin down.
3. Use the supernatant as the sample for Native-PAGE.

Sample Preparation for SDS-PAGE

1. Add an equal volume of **EzApply** (**AE-1430**) or 2x SDS sample treatment solution to the protein solution and mix thoroughly.
2. Heat the mixture at 100°C for 10 minutes.
3. Centrifuge at 14,000 x g for 10 minutes (4°C).
4. Use the supernatant as the sample for SDS-PAGE.

11. ATTO related products

Native-PAGE sample preparation kit
WSE -7011 EzApply Native

SDS-PAGE sample preparation kit
AE -1430 EzApply

2D electrophoresis sample preparation kit
AE- 1435 EzApply 2D kit

Native PAGE running buffer
WSE- 7055 EzRun T.G.

High Resolution Clear Native PAGE running buffer
WSE- 7056 EzRun ClearNative

Blue Native PAGE running buffer
WSE- 7057 EzRun Blue Native

Native Molecular weight markers for PAGE
WSE -7016 EzStandard Native

phosphate-buffered saline
WSE- 7430 EzPBS (-)

Even when following the same protocol, small variations in technique may result in large differences in outcomes. For detailed experimental tips, please visit the ATTO website (https://www.atto.co.jp/) to download our know-how resources.

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