WSE-7420 EzRIPA Lysis kit

February 6, 2013: The 2nd version

1. Instructions for ensuring the safe use of this Product

Please read this Instruction Manual thoroughly to ensure the safe use of the Product. Do not use the Product unless you fully understand the contents of this Instruction Manual. The Instruction Manual describes the method and purpose of use intended for the Product. Please refrain from using it for the purpose and method not described in the Instruction Manual. If the Product should be used for the purpose and method not described in the Instruction Manual, the user must be responsible for any necessary safety measures and any unpredictable consequences.
Also, please read thoroughly the instruction manuals for the devices to be used together with the Product.

2. Purpose of use

The EzRIPA Lysis kit is designed to dissolve mammalian cell culture and prepare protein extracts. By using the Protease Inhibitor and Phosphatase Inhibitor included in the Product, this kit can dissolve protein while preventing protein decomposition and dephosphorylation. Also, the protein extracts can be used for electrophoresis, immunoprecipitation, ELISA and other biochemical/immunological analysis. As this solution is HEPES base buffer and does not contain amino or phosphate group, it can be used as it is for protein marking experiment and other appropriate experiments.

3. Product configuration

Name	Content	Piece(s)	Storage
RIPA Lysis buffer	100 mL	1	4 °C
Protease Inhibitor	1 mL	1	- 20 °C
Phosphatase Inhibitor	1 mL	1	- 20 °C

4. Composition

Name	Principal elements
RIPA Lysis buffer	20 mM HEPES, 150 mM NaCl, 1.0% IGEPAL® CA-630, 0.1% SDS, 0.5% Sodium deoxycholate/ pH7.5
Protease Inhibitor	100 × concentration, aprotinin, pepstatin A, leupeptin, DMSO
Phosphatase Inhibitor	100 × concentration, sodium fluoride, sodium vanadate, sodium glycerophosphate

IGEPAL® CA-630 is the same material as Nonidet P-40. IGEPAL® is the registered trademark of Rhone-Poulenc AG Co.

This Product does not contain any poisonous and deleterious substances under the Poisonous and Deleterious Substances Control Act or any substances that are to be notified and exceed the exemption amount under the Industrial Safety and Health Act. Matters that are to be notified and exceed the exemption amount for the specified chemicals under the PRTR Act are partially included. Please download the MSDS of this Product from our website (http://www.atto.co.jp/) to check details.

5. Storage

- *RIPA Lysis buffer (**R**adio-**I**mmuno**p**recipitation **a**ssay Lysis buffer)* should be kept in cold storage (2 - 8 °C). Unless it is opened, it will be stable until the expiration date.
- *Protease Inhibitor* and *Phosphatase Inhibitor* should be kept in frozen storage (-20 °C). Unless it is opened, it will be stable until the expiration date.

6. Disposal

Please comply with the disposal method of your organization when disposing reagents.

7. Required items other than this Product

- Ice cold PBS buffer
- Micro-centrifuge tube
- Vortex mixer
- Seesaw shaker or rotator
- Cooled centrifuge (Micro-centrifuge tube)

8. Precaution on use

- This Product is delivered by refrigerated goods transportation. Please open the package immediately after receiving and store the Product under the temperature suitable for each reagent.
- Please ice-cool all reagents before you start the experiment. Also, please process the experiment and operation on the ice or at low temperature.
- As the Protease Inhibitor includes DMSO, it may be frozen at low temperature. Please thaw it completely at room temperature before use.
- Please increase or decrease the amount for adding the Protease Inhibitor or add AEBSF, Bestatin or other inhibitors as needed.

9. How to use

9.1. Preparation of the *RIPA Lysis buffer*

1. Mix the Protease Inhibitor and Phosphatase Inhibitor as needed, in accordance with the table below. Place the RIPA Lysis buffer after mixing on the ice until it is used.

Content	Protease Inhibitor (blue lid)	Phosphatase Inhibitor (opaque)
1 mL	(10 µL)	(10 µL)

*The amount of RIPA Lysis buffer necessary for cell solubilization is approximately 1 mL for the cells of 5×10^6 - 2×10^7.

9.2. Solubilization of cultured cells

(1) For adherent cells

1. Add the appropriate amount of ice cold PBS to the culture dish where cells are cultured. Then, wash the cells twice or more.
2. Add the RIPA Lysis buffer prepared in 9.1. Preparation of the RIPA Lysis buffer to the culture dish for the cells washed with PBS.
   *The amount of RIPA Lysis buffer necessary for cell solubitization is approximately 1 mL for the cells of 5×10^6 - 2×10^7.
3. Incubate them on the ice for 15 minutes.
4. Scrape the cells from the culture dish with a scraper and transfer them to the micro-centrifuge tube.
5. Centrifuge at 14,000 × g for 5 - 15 minutes.
6. Transfer the centrifugal supernatant to a new micro-centrifuge tube. Place the solubilized protein solution on the ice or store it at – 80 °C till it is used.

(2) For suspension culture cells or cells collected by the trypsin treatment

1. Prepare cell suspension collected by the trypsin treatment or other appropriate method.
2. Centrifuge the cell suspension at 200 × g for 3 - 5 minutes.
3. Carefully remove and dispose the centrifugal supernatant. Then, add the appropriate amount of ice cold PBS to the cells (centrifugal sediment) and suspend them.
4. Centrifuge the cell suspension at 200 × g for 3 - 5 minutes.
5. Carefully remove and dispose the centrifugal supernatant. Then, add the appropriate amount of ice cold PBS to the cells (centrifuge sediment) and suspend them.
6. Centrifuge the cell suspension at 200 × g for 3 - 5 minutes.
7. Remove and dispose the centrifugal supernatant and add the RIPA Lysis buffer prepared in I. Preparation of the RIPA Lysis buffer to the cells (centrifugal sediment). Then, suspend them by pipetting.
   *The amount of RIPA Lysis buffer necessary for cell solubitization is approximately 1 mL for the cells of 5×10^6 - 2×10^7.
8. Incubate on the ice for 15 minutes.
   *During incubation, invert them two or three times every one - two minutes.
9. Centrifuge the cell solution at 14,000 × g for 5 - 15 minutes.
10. Transfer the centrifugal supernatant to a new micro-centrifuge tube. Place the solubilized protein solution at rest on the ice or store it at – 80 °C until it is used.
    *Efficiency of nuclear protein extraction will increase by adding SDS to obtain the final concentration of 0.5%. As the viscosity increases due to DNA elution, process ultrasonic fragmentation or treatment with DNase I (100 - 500U/mL).

10. Reference

Sample preparation for SDS-PAGE

1. Add the EzApply (AE-1430) of the same amount as that of protein solution or add 2 × SDS sample treatment liquids and mix them well.
2. Process heating of the mixed solution at 100 °C for 10 minutes.
3. Centrifuge at 14,000 × g for 10 minutes (4 °C).
4. The centrifugal supernatant is used for the sample for SDS-PAGE.

11. Related ATTO products

- SDS-PAGE sample preparation kit
  AE-1430: EzApply
- Sample preparation kit for 2D electro-phoresis
  AE-1435: EzApply 2D kit
- Organelle extraction kit
  WSE-7421: EzSubcell Extract
- Organelle fraction kit
  WSE-7422: EzSubcell Fraction
- SDS removal reagent
  AE-1390: SDS-eliminant
- Electrophoresis equipment with a built-in power supply
  WSE-1020: Compact PAGE / Twin-R
  WSE-1100: PageRun-R

*The experiment operation may have a significant variance in the results due to a slight technical difference in the same protocol. It is important to know the "Tips" to obtain the optimal result.
As our website provides various "tips on experiment" and you can download the document, please visit our website below and read the article.
http://www.atto.co.jp/

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