Fermentas
LIFE SCIENCES

CERTIFICATE OF ANALYSIS

ProteoJET™ Mammalian Cell Lysis Reagent

#K0301 250 ml
Lot:

Store at room temperature or at 4°C.

ISO 9001 ISO 14001
www.fermentas.com

Description

ProteoJET™ Mammalian Cell Lysis Reagent is a ready-to-use solution designed for highly effective and convenient total protein extraction from any mammalian cultured cells or tissue samples at room temperature. The procedure is fast and simple: no sonication and no freeze-thaw cycles are required.
Proteins extracted using this reagent are non-denatured, functionally active and therefore ideal for direct use in many common downstream applications, including 1D and 2D electrophoresis, Western blotting, electrophoretic mobility shift assay (EMSA), immunoprecipitation, affinity purification, enzymatic activity and reporter gene assays. Isolated proteins are compatible with quantification assays such as Bradford, Lowry and the BCA assay.

The reagent provided is sufficient for:
– 125 extractions from 100 µl of wet cell pellet or
– 500 extractions from 100 mg of tissue.

Additional Materials Required

- Protease inhibitor cocktail
- Microcentrifuge tubes
- Vortex mixer
- Shaker
- Microcentrifuge
- PBS (137 mM NaCl, 2.7 mM KCl, 100 mM Na_2HPO_4, 2 mM KH_2PO_4, pH 7.4)

Important Notes

- Protease inhibitor cocktail, e.g., ProteoBlock™ Protease Inhibitor Cocktail #R1321, may be added to ProteoJET™ Mammalian Cell Lysis Reagent to minimize proteolysis.
- The volume of the ProteoJET™ Mammalian Cell Lysis Reagent used depends on the amount of cells and on the expected final protein concentration in the extract. The following recommendations can be considered for general guidance:

Type of sample	Volume of ProteoJET™ Mammalian Cell Lysis Reagent
Suspension cells $5x10^6$ cells	200 µl (20 volumes of lysis reagent to 1 volume of packed cells)
Adherent cells 35 mm plate 100 mm plate	 400 µl 1000 µl
Tissue 100 mg	 500 µl

QUALITY CONTROL

The ProteoJET™ Mammalian Cell Lysis Reagent is tested using the protein extraction protocol with suspension cells. Extracted proteins are separated on 12% SDS-PAGE, transferred to PVDF membrane and blotted for the presence of tubulin.

Quality authorized by:  Jurgita Zilinskiene

PRODUCT USE LIMITATION.

This product is developed, designed and sold exclusively *for research purposes and in vitro use only*. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Please refer to www.fermentas.com for Material Safety Data Sheet of the product.

(see reverse page for PROTOCOLS)

PROTOCOLS

For adherent cells

1. Remove the growth medium from the cells.
2. Rinse cells once with PBS to remove residual medium. Discard washing buffer.
3. Cell lysis:

For direct lysis in culture plates

- Add an appropriate volume of ProteoJET™ Mammalian Cell Lysis Reagent (400 µl for 35 mm plate or 1000 µl for 100 mm plate).
- Incubate for 10 minutes at room temperature on a shaker.
- Collect lysate from plates into a microcentrifuge tube (to maximize recovery, scrape cell debris using cell scraper).

For lysis in a microcentrifuge tubes (generates protein extracts at higher concentration)

- Collect cells by scraping in appropriate volume of PBS or by trypsinization.
- Transfer cells to a microcentrifuge tube.
- Pellet cells by centrifugation at 250 x g for 5 minutes and discard supernatant. Estimate the packed cell volume.
- Add 20 volumes of ProteoJET™ Mammalian Cell Lysis Reagent to 1 volume of packed cells. Resuspend cell pellet by vortexing.
- Incubate for 10 minutes at room temperature on a shaker (900-1200 rpm).

4. Clarify the lysate by centrifugation at 16,000-20,000 x g for 15 minutes.
5. Transfer the supernatant to a new tube. Use the cell lysate immediately or store at -70°C for future use.

For suspension cultured cells

1. Collect cells in an appropriate centrifuge tube.
2. Pellet cells by centrifugation at 250 x g for 5 minutes and discard the supernatant.
3. Rinse cells once with PBS to remove residual medium and repeat centrifugation step. Estimate the packed cell volume.
4. Add 20 volumes of ProteoJET™ Mammalian Cell Lysis Reagent to 1 volume of packed cells. Resuspend cell pellet by vortexing.
5. Incubate for 10 minutes at room temperature on a shaker (900-1200 rpm).
6. Clarify the lysate by centrifugation at 16,000-20,000 x g for 15 minutes.
7. Transfer the supernatant to a new tube. Use the cell lysate immediately or store at -70°C for future use.

For tissue cells

1. Weigh out the tissue sample.
2. Transfer (either fresh or frozen) to a mortar and crush the tissue in liquid nitrogen with a pestle. During homogenization keep the tissue completely frozen to preserve functional and structural integrity of proteins.
   Note. Complete tissue homogenization is a critical step for total protein yield.
3. Transfer the tissue powder to a microcentrifuge tube and add an appropriate volume of ProteoJET™ Mammalian Cell Lysis Reagent (500 µl for 100 mg of tissue).
4. Resuspend tissue powder by vortexing and incubate for 10 minutes at room temperature on a shaker (900-1200 rpm).
5. Clarify the lysate by centrifugation at 16,000-20,000 x g for 15 minutes.
6. Transfer the supernatant to a new tube. Use the cell lysate immediately or store at -70°C for future use.

TROUBLESHOOTING

Problem	Cause & Solution
Low protein yield	Insufficient volume of cell lysis reagent used. Add more cell lysis reagent.
	Insufficient dispersion of cells. Thoroughly vortex pelleted suspension cells following centrifugation. Use non-confluent cells. Adherent cells should be removed from the culture dish with a cell scraper or by trypsin treatment. Homogenize tissues thoroughly.
	Insufficient cell lysis. Prolong incubation with cell lysis reagent and shake more vigorously during incubation.
Low protein concentration	Excessive volume of lysis reagent used. Decrease the volume of lysis reagent or increase number of cells.
Protein activity is absent or low	Proteins are degraded. Limit procedure time to a minimum and freeze samples immediately after extraction. Use protease inhibitor cocktail, e.g., ProteoBlock™ Protease Inhibitor Cocktail #R1321.