ab113475 – Whole Cell Extraction Kit

Instructions for Use

For the extraction of cellular proteins from mammalian cells and tissue samples

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

Version 1 Last Updated 17 September 2019

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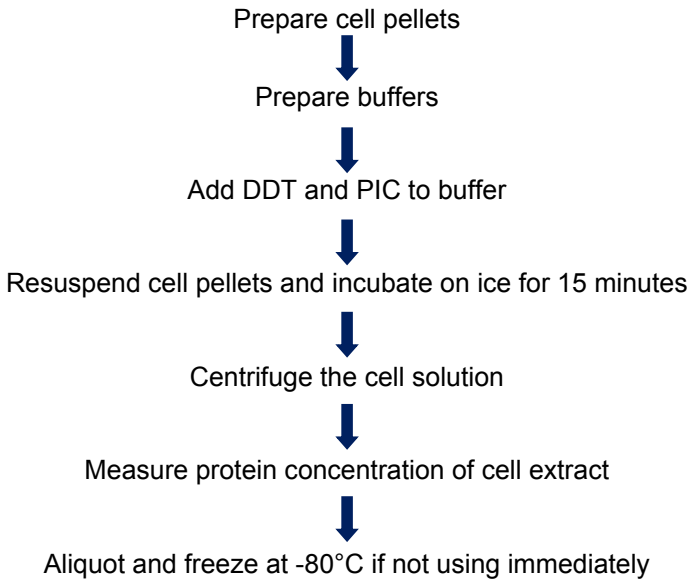
1. BACKGROUND

Abcam's Whole Cell Extraction Kit provides a simple and selective method for extracting whole cell proteins used for a variety of applications. These applications may include western blotting, cellular enzyme assays, and others requiring cellular proteins. ab113475 can be used to extract cellular proteins from mammalian cells and tissue samples. The extraction procedure can be completed within 45 minutes.

2. ASSAY SUMMARY



3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit as given in the table upon receipt.

Observe the storage conditions for individual prepared components in sections 9 & 10.

For maximum recovery of the products, centrifuge the original vial prior to opening the cap.

5. MATERIALS SUPPLIED

Item	100 Tests	Storage Condition (Before Preparation)
5X Extraction Buffer	20 mL	4°C
1000X DTT Solution	100 µL	4°C
1000X Protease Inhibitor Cocktail (PIC)	100 µL	4°C

6. MATERIALS REQUIRED, NOT SUPPLIED

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

- Microcentrifuge vials
- Pipettes and pipette tips
- Distilled water
- Hemacytometer
- 15 mL conical tubes
- Vortex
- Ice

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures
- Do not use kit or components if it has exceeded the expiration date on the kit labels
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding

8. TECHNICAL HINTS

- Avoid foaming or bubbles when mixing or reconstituting components
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions
- Ensure plates are properly sealed or covered during incubation steps
- Complete removal of all solutions and buffers during wash steps
- **This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions**

9. REAGENT PREPARATION

All reagents provided are ready to use.

10. SAMPLE PREPARATION

10.1 **Monolayers or Adherent Cells:**

10.1.1 Cells (treated or untreated) are grown to 80-90% confluency and trypsinized after removing growth medium. Cells are then collected into a 15 mL conical tube and counted in a hemacytometer.

10.1.2 Cells are washed once with PBS and pelleted by centrifugation at 1000 rpm for 5 minutes.

10.2 **Non-adherent Cells:**

10.2.1 Grow cells to 2 x 10^6/mL and collect the cells into a 15 mL conical tube.

10.2.2 Centrifuge the cells for 5 minutes at 1000 rpm and discard the supernatant. Wash cells with PBS once by centrifugation at 1000 rpm for 5 minutes. Discard the supernatant.

11. ASSAY PROCEDURE

11.1 Prepare 1X Extraction Buffer by adding 1 mL of the 5X Extraction Buffer to 4 mL of distilled water.

11.2 Add 1000X DTT Solution and PIC to 1X Extraction Buffer at a 1:1000 ratio. Re-suspend cell pellet in 100 µL of ice cold 1X Extraction Buffer per 10^6 adherent cells, or 2 x 10^6 non-adherent cells.

11.3 Transfer the cell solution to a micro-centrifuge vial. Incubate on ice for 15 minutes with vigorous vortex (5 seconds) per 5 minutes.

11.4 Centrifuge the cell solution for 10 minutes at 14,000 rpm at 4ºC and transfer the supernatant into a new microcentrifuge vial.

11.5 Measure the protein concentration of cell extract.

11.6 Use immediately or aliquot and freeze supernatant at -80ºC until further use. Avoid freeze/thaw cycle.

12. NOTES

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