

Version 2a Last updated 1 March 2023

ab270054

Protein Extraction Kit

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Protein Extraction Kit:

www.abcam.com/ab270054

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For the the solubilization of proteins for a variety of proteomics applications.

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

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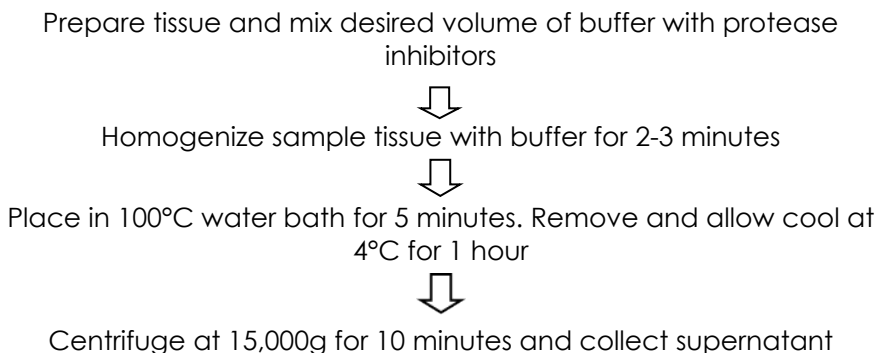
1. Overview

Protein Extraction Kit (ab270054) is for the solubilization of proteins for a variety of proteomics applications. The kit contains a proprietary buffer designed to maximize the extraction of proteins from cells and tissues under conditions that are compatible with downstream proteomics applications such as mass spectrometry (MS). The Protein Extraction buffer contains reducing agents and SDS and provides unbiased extraction of proteins from cells and tissues. The resulting lysates are compatible with protein quantification assays, fractionation using the Gelfree® 8100 Sample Fractionation System, other SDS-PAGE separations, and the Filter-Aided Sample Preparation (FASP) method.

The kit provides efficient protein extraction for both freshly harvested cells and frozen cells. It works with high efficiency on a wide variety of samples such as heart, liver, brain, and kidney organ tissues, mammalian derived cultured cell lines, and bacteria.

The Protein Extraction buffer contains SDS and reducing agents. For accurate determination of total protein concentration, use an assay that is compatible with detergent and reducing agent.

The kit is designed for maximum, unbiased extraction, reduction, and denaturation of whole proteomes. It should not be used prior to functional protein assays.



2. Materials Supplied and Storage

Store Protein Extraction Buffer at 4°C. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Item	30 tests	Storage temperature (before prep)
Protein Extraction Buffer	50 mL	4°C

3. Materials Required, Not Supplied

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

- Protease inhibitor cocktail
- Dounce homogenizer
- Water bath or hot plate set to 100°C

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:

www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

When preparing tissue for proteomics analysis, it is recommended that Protein Extraction Buffer be used in conjunction with protease inhibitors. Both freshly harvested cells and frozen cells can be used.

Protease inhibitors:

Protease Inhibitor Cocktail EDTA Free (3, 10 and 30 mL) – ab270055

Protease Inhibitor Cocktail EDTA Free (1 mL) – ab201111

Protease Inhibitor Cocktail (3, 10 and 30 mL) – ab271306

Protease Inhibitor Cocktail (1 kit) – ab65621

6. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature just prior to use and gently agitate.

- 6.1 Weigh out a portion of tissue.
- 6.2 Measure out a volume of Protein Extraction Buffer at a ratio of 10 mL buffer per 1g of tissue, or 0.3 g of wet cell pellet.
- 6.3 Add protease inhibitors to the Protein Extraction Buffer.
- 6.4 Add a volume of mixed buffer consisting of Protein Extraction Buffer and protease inhibitors at a ratio of 10 mL mixed buffer per 1g of tissue, or 0.3 g of wet cell pellet.
- 6.5 Homogenize the sample tissue with buffer in a dounce homogenizer for two to three minutes.
- 6.6 Place the sample in a 100°C water bath for five minutes.
- 6.7 Remove the sample from the water bath and allow it to cool at 4°C for one hour.
- 6.8 Centrifuge the sample at 15,000 $\times g$ for 10 minutes to pellet the cellular debris.
- 6.9 Collect the supernatant for analysis.

Technical Support

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