

## Protein Extraction Kit

Catalog #: EXT020

20 Reactions



## INTRODUCTION

The Protein Extraction Kit is a great tool for preparation and isolation of total proteins. The kit contains optimized reagents and materials, which efficiently extract and isolate proteins from any cells and tissues. The Lysis Beads enable effective and rapid lysis of cells and tissues to allow complete release of proteins. The total proteins isolated by this kit can be used for a variety of applications, including protein microarray assays, Western blots/immunoprecipitation, gel mobility shift assays, and other procedures.

## FEATURES

- Extract proteins from cells or tissues
- Allow efficient release of proteins to maximize yield
- Easy to use protocol

## EXPERIMENTAL CONSIDERATIONS

- All reagents and materials are intended for research use only.
- Always wear gloves before handling any reagents.
- Use extra care. Any variation in buffers, operator, pipetting technique, washing technique, and incubation time or temperature can alter the performance of the kit.
- Only use reagents and materials recommended by this user's guide. Do not substitute buffers or solutions from other sources.
- The Protein Extraction Kit does not include protease inhibitors. To prevent protein degradation, once you start the extraction, you should work quickly and proceed diligently towards the array analysis step. If you plan to store the protein extract for a longer period of time (one week), you may add protein inhibitors accordingly.

## COMPONENTS

| Material/Reagent  | Quantity | Purpose               | Storage Condition |
|-------------------|----------|-----------------------|-------------------|
| Extraction Buffer | 10 mL    | Protein extraction    | 4 °C              |
| Lysis Beads       | 20 tubes | Cell and tissue lysis | 4 °C              |

**ADDITIONAL MATERIAL REQUIRED**

- 1X PBS
- BCA Protein Assay Kit (Pierce, Cat. #: 23227)
- Ice
- Vortexer
- Centrifuge

**PROTOCOL****A. Protein Extraction from Cells**

1. Harvest the cells by centrifugation.
2. Wash the cells with ice cold 1X PBS and centrifuge at 4°C. Aspirate and discard the supernatant.
3. Wash the cells two more times with ice cold 1X PBS and centrifuge at 4°C.
4. Add one tube of Lysis Beads to the cell pellet that contains a maximum 25 million cells.
5. Add 50 to 250  $\mu$ L of Extraction Buffer. Mix rigorously by vortexing for 30 seconds to 1 minute. Incubate the mixture on ice for 10 minutes.
6. Repeat vortexing for 30 seconds to 1 minute at 10-minute intervals for 1 hour. Be sure to incubate the mixture on ice between vortexing.
7. Centrifuge the mixture at 10,000  $\times$  g for 30 minutes at 4°C.
8. Transfer the supernatant to a clean tube. Discard the beads.
9. Measure the protein concentration using BCA Protein Assay Kit.
10. Proceed immediately to protein labeling/array processing, or store the extracts at -80°C. You may consider adding protease inhibitors if you plan to store the extracts for a long period of time.

**B. Protein Extraction from Tissues**

1. Add one tube of Lysis Beads to tissues (up to 1g of tissues).
2. Add 50 to 250  $\mu$ L of Extraction Buffer. Mix by vortexing for 30 seconds to 1 minute. Incubate the mixture on ice for 10 minutes.
3. Repeat vortexing for 30 seconds to 1 minute at 10-minute intervals for 1 hour. Be sure to incubate the mixture on ice between vortexing.
4. Centrifuge the mixture at 10,000  $\times$  g for 30 minutes at 4°C.
5. Transfer the supernatant to a clean tube. Discard the beads.
6. Measure the protein concentration using BCA Protein Assay Kit.
7. Proceed immediately to protein labeling/array processing, or store the extracts at -80°C. You may consider adding protease inhibitors if you plan to store the extracts for a long period of time.