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Mammalian Active Protein Extraction Reagent

1 Packing list

Components	HY-K1003-100 mL	HY-K1003-500 mL
Mammalian Active Protein Extraction Reagent	100 mL	100 mL × 5

2 Introduction

MCE Mammalian Active Protein Extraction Reagent are gentle, non-denaturing detergents designed for the rapid, high-quality, and high-activity extraction of cytoplasmic, nuclear, and membrane proteins from mammalian cells or tissues.

Features of MCE Mammalian Active Protein Extraction Reagent
Gentle: Yielding extracts that are immediately compatible with coomassie (Bradford) and BCA protein assays or SDS-PAGE;
Compatible: The extracted soluble proteins are in non-denatured state, which can maintain its original spatial structure and biological activity, and can be directly used in immunoassays, enzyme analyses and other affinity purification procedures;
Maintain activity: Maintain activity of luciferase, beta-galactosidase, CAT and other reporter genes.

3 General Protocol

Experiment Preparation

Prepare an appropriate quantity of MCE Mammalian Active Protein Extraction Reagent and supplement it with Protease Inhibitor (MCE Cat No. HY-K0010，HY-K0011) and Phosphatase Inhibitor (MCE Cat No. HY-K0021) according to the experimental needs. Set aside on ice prior to the experiment.

1. Procedure for protein extraction of adherent-cultured mammalian cells
   (1) Cell Collection: Carefully remove culture medium from adherent cells.
   (2) Cell Lysis: Add the appropriate amount of MCE Mammalian Active Protein Extraction Reagent (see Table 1), mix gently and incubate on ice for 2-10 min.

Table 1. The volume of Extraction Reagent to use for different sizes of standard culture plates.

Plate Size	Extraction Reagent Volume (μL)
100 mm	500-1000
60 mm	200-400
6-well	100-200
12-well	50-100
24-well	25-50
48-well	12.5-25
96-well	5-10

(3) Lysate Collection: Collect the lysate and transfer to a tube. Centrifuge at 14,000 × g for 5-10 min at 4°C to pellet the cell debris.

(4) Protein Collection: Transfer the supernatant to a new tube for analysis or store at -80°C.

Note: a. If the culture medium contained phenol red or other reagents that could interfere with subsequent protein analysis, wash cells 1-2 times in PBS and discard the supernatant.

b. The incubation time can be adjusted according to the complexity of protein extraction in order to yield more protein.

2. Procedure for protein extraction of suspension-cultured mammalian cells

(1) Cell Collection: Centrifuge the cell suspension at 2,500 g for 10 min at 4°C, discard the supernatant.

(2) Cell Lysis: Gently tap the bottom of the centrifuge tube to adequately disperse the cell pellet, add appropriate amount of MCE Mammalian Active Protein Extraction Reagent (see Table 1), mix gently and incubate on ice for 2-10 min.

(3) Lysate Collection: Centrifuge at 14,000 × g for 5-10 min at 4°C.

(4) Protein Collection: Transfer the supernatant to a new tube for analysis or store at -80°C.

Note: a. If the culture medium contained phenol red or other reagents that could interfere with subsequent protein analysis, wash cells 1-2 times in PBS and discard the supernatant.

b. The incubation time can be adjusted according to the complexity of protein extraction in order to yield more protein.

3. Procedure for protein extraction of tissues

(1) Tissues pre-treatment: Cut the tissue into small pieces, or freeze it with liquid nitrogen and grind it into a powder.

(2) Tissue Homogenization: Add MCE Mammalian Active Protein Extraction Reagent at a ratio of 20 mg of tissue to 200-400 µL of Extraction Reagent. Homogenize the tissues to ensure complete lysis, and incubate on ice for 2-10 minutes.

(3) Homogenization Collection: Centrifuge at 14,000 × g for 5-10 min at 4°C.

(4) Protein Collection: Transfer the supernatant to a new tube for analysis or store at -80°C.

Note: The incubation time can be adjusted according to tissue types.

4 Storage

4°C，2 years

5 Precautions

1. All steps of protein extraction should be executed on ice or at 4°C to ensure the preservation of protein activity.
2. When extracting His-tagged recombinant proteins for purification processes, it is recommended to avoid the use of reagents that contain EDTA.
3. The detergent in the extraction reagents can be removed by dialysis or ultrafiltration.
4. The cells will start to lyse after the extraction reagent is added, and the appearance of a viscous gel-like substance is normal. Adding nuclease to the extraction reagent can reduce this viscosity, and the amount of the extraction reagent can also be increased.
5. It is recommended to avoid contacting the precipitate during supernatant aspiration to prevent the introduction of impurity proteins.
6. The precipitates from cell lysis can be retained for subsequent verification analysis.
7. This product is for R&D use only, not for drug, household, or other uses.
8. For your safety and health, please wear a lab coat and disposable gloves to operate.