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User's Manual and Instructions

Membrane Protein Extraction Kit

Catalog Number: K3014005, K3014005-1, K3014005-2

Introduction

BioChain Membrane Protein Extraction Kit only contains two reagents, Cytoplasm protein remove buffer (Buffer C) and Membrane protein extraction buffer (Buffer E). The Membrane Protein Extraction kit is able to extract membrane protein from mammalian tissues and cells based on a proprietary technique. Preparation of membrane protein extracts using this kit can be done quickly and efficiently. First, tissues or cells are homogenized in the Cytoplasm protein remove buffer and then remove the Cytoplasm protein by centrifuging. The membrane protein can be easily isolated from the pellet by using the Membrane Protein Extraction Buffer extraction. This procedure is suitable for a variety of tissues and cultured cells.

Features

- Reliable - Super-quality and highly reproducible separation of membrane proteins from variety samples
- Fast – isolate the membrane proteins in less than two hours
- Pure - Minimal cross contaminations
- Simple - Easy to use comparing to other methods, such as differential centrifugations

Applications

- Membrane protein expression
- Enrichment of low-abundance proteins or enzymes for activity or function assay
- Protein- protein interaction studies of membrane proteins.
- Receptors function study

Description

Components in this kit are prepared with pure chemicals according to the proprietary technology. To prevent protein degradation, a ready-to-use protease inhibitor cocktail is provided. BioChain's Membrane Protein Extraction Kit is designed to quickly isolate membrane proteins from mammalian tissues and cells. The kit is consisted of reagents enough to enrich membrane proteins from 5 grams tissues or about 125 million cells. The protein product has been investigated by SDS-PAGE and immunoblotting of selected marker proteins.

Quality Control

The kit of this lot has been tested to go through the membrane protein extraction procedure from rat colon. The proteins from the extraction are used for electrophoresis, transferring to PVDF membrane and immunoblotting with Mouse anti-Na/K ATPase as primary antibodies, and HRP-conjugated anti-Mouse IgG as secondary antibodies.

Contents

Membrane Protein Extraction Kit

Item	Component	Amount	Part No.
Buffer C	HEPES (pH7.9), $MgCl_2$, KCl, EDTA, Sucrose, Glycerol, Sodium OrthoVanadate	22 ml	K3014005-1
Buffer E	Tris-HCl (pH7.5), Trito X-100, Glycerol, NaCl	6 ml	K3014005-2
50 x PI	A cocktail of protease inhibitors	0.6 ml	
Note	For ordering 50 x PI separately, please find Cat# K3011010-2		

Reagents are sufficient for extraction of membrane proteins from 5 g tissue or about 125 million cells. Minimal 0.1 g tissue or 2.5 million cells should be required.

Storage and Stability
Buffers are stored at 2-8 °C. The 50 x PI solution should be stored at -20 °C. If the kit is going to be used for multiple extractions, aliquot the PI solution properly before storing. The kit is stable for 1 year when handled properly.

Reagents and Equipments not provided
- Tissue Homogenizer
- Centrifuge and microcentifuge
- Rolling facility

Protocol

For buffer C and E, **add 50xPI to working concentration (1 x) before usage**.

1. Weigh certain amount of tissue (Wt. gram), chop it to small pieces, and then pipette ice cold buffer C at **2.0 ml per gram tissue or per 20 million cells**. Homogenize the tissue or cells at moderate speed (e.g., speed 4) for 20 sec. Let it stand on ice for a few seconds, repeat homogenization twice.
2. Rotate the mixture at 4°C for 20 min. Spin at 18,000 g at 4°C for 20 min. Remove the cytoplasmic proteins (the supernatant).
3. Resuspend the pellet with Wt (g) x 2.0 ml ice cold buffer C; rotate at 4°C for 5' min. Spin at 18,000 g at 4°C for 20 min. Drain the supernatant.
4. Add Wt (g) x 1.0 ml ice cold buffer E to resuspend pellet from step 3, rotate at 4°C for 20 min. Spin at 9,000 g at 4°C for 20 min. The membrane proteins are in the supernatant,
5. Measure the protein concentration. Aliquot and label the proteins properly, store at –70°C.

Trouble Shooting

1. Cytoplasmic proteins-carryover to membrane fractions
Repeat the washing step (step 3 in the protocol) 1-2 times to completely remove cytoplasmic proteins.

2. Protein degradations
Make sure PI was added to all of the Buffers before use.
50 x PI was not aliquot, and lost activity when frozen-thawed for too many times.
Homogenize the tissue for less time (< 20 sec), let the tube stand on ice for a few seconds before next homogenization

Related Products
Compartmental proteins, Total Proteins, Total Protein Extraction Kit

Reference
1. Neelam Sharma-Walia et al *J. Virol.,* Apr 2004; 78: 4207 – 4223
2. Christian Korn et al *J. Biol. Chem.,* Nov 2004; 10.1074/jbc.M413035200