ACExtract Nuclear and Cytoplasmic Protein extraction kit

Cat# CE1009 – 50 rxn / CE1010 – 100 rxn

Storage at -20 ℃ for one year

INTRODUCTION

ACExtract Nuclear and Cytoplasmic Protein extraction kit provides a simple and convenient method for extracting nuclear protein and cytoplasmic proteins from cells to fresh tissues. The operating procedure is completed within 90 minutes.

The kit uses the cytoplasmic solution A and B to damage the cells through osmotic pressure, releasing the cytoplasmic protein. The lysate is then centrifuged to obtain supernatant (cytoplasmic protein) and pellet (nuclear protein). Finally, the nuclear protein is obtained by extraction with a high-salt nuclear solution C.

CONTENTS

No	Component	CE1009 – 50 rxn	CE1010 – 100 rxn	Storage Condition
FA	Cytoplasmic Reagent A	25 ml	25 ml x 2	4℃
FB	Cytoplasmic Reagent B	1.5 ml	3.0 ml	4℃
FC	Nuclear Reagent C	12.5 ml	25 ml	4℃
FD	DTT	50 µl	100 µl	-20℃
FE	Proteinase Inhibitor	250 µl	500 µl	-20℃
FF	PMSF (100 mM)	400 µl	800 µl	-20℃

PROTOCOL

Note : In order to ensure intact extraction, the procedure should be operated at low temperature and doing as soon as possible.

1. Sample Preparation

a. Cell

1. Harvest cell by centrifugation at 2000 rmp, 5min.
2. Discard the supernatant and suspend cell with PBS.
3. Estimate 5 x 10^6 - 1x10^7 cell number and pellet by centrifugation at 2000 rmp, 5 min.

b. Tissue

1. Weight 100-500 mg fresh tissue and wash with PBS.
2. Tear tissue into pieces with scissors and transfer to new microcentrifuge tube.
3. After homogenizing tissue with adequate cold PBS, place steady for 5 min and centrifuge at 2000 rpm, 5 min.
4. Discard the supernatant and estimate the paced cell volume (PCV).

Suggested reagent volumes for packed cell volume (PCV)			
Packed Cell Volume (PCV)	Cytoplasmic Reagent A	Cytoplasmic Reagent B	Nuclear Reagent C
10 µl	100 µl	5.5 µl	50 µl
20 µl	200 µl	11 µl	100 µl
50 µl	500 µl	27.5 µl	250 µl
100 µl	1 ml	55 µl	500 µl

4. Reagent Preparation

1. Add 1 µl DTT, 5 µl PMSF and 5 µl Proteinase Inhibitor per 1 ml Cytoplasmic Reagent A.
2. Add 1 µl DTT, 5 µl PMSF and 5 µl Proteinase Inhibitor per 1 ml Cytoplasmic Reagent C.

Note : Due to unstable of PMSF in aqueous solution, PMSF should be prepared 3 min before adding into sample.

5. Cytoplasmic and Nuclear Protein Extraction

1. Add 200 µl pre-cooled Cytoplasmic Reagent A into 20 µl cell pellet obtained from step1 and vortex vigorously for 10 sec. Place one ice for 10 – 15 min.

Note : If the cell pellet is not completely suspended ,the vortex time can be extended.

2. Add 11 µl pre-cooled Cytoplasmic Reagent B and vortex vigorously for 5 sec. Place one ice for 1 min.
3. Vortex vigorously for 5 sec again and then centrifuge at 4℃, 13000 rpm, 5min.
4. Immediately transfer the supernatant into a pre-cooled plastic tube, which is the extracted cytoplasmic protein. It can be used immediately or frozen.

Note : Do not disturb the sediment and let a little supernatant retained for avoiding contact with precipitate.

5. For the precipitation, add 100 µL of pre-cooled Nuclear Reagent C to the pellet and vortex vigorously for 15 sec. Place one ice for 30 min.
6. Continue vortex for 15 sec every 3 min and then centrifuge at 4℃, 13000 rpm, 10min.
7. Immediately transfer the supernatant into a pre-cooled plastic tube, which is the extracted nuclear protein. It can be used immediately or frozen.
8. The extracted cytoplasmic and nuclear are quantified BCA or Bradford method and stored at -80 °C, avoiding repeated freezing and thawing.

PRODUCT USE LIMITATION

These products are intended for research use only.