Experiment Protocol 402

Preparation of cell extract (Small scale method)

1. Materials

1.1 Cell

- Cultured cells(Floating cells, Adhesive cells) >10^7 cells

1.2 Reagents

- PBS(-)
- 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES)
- Sodium hydroxide (NaOH)
- Potassium chloride (KCl)
- Magnesium chloride ($MgCl_2$)
- Sodium chloride (NaCl)
- Calcium chloride ($CaCl_2$)
- Ethylenediamine tetraacetic acid (EDTA)
- Glycerol (Glycerin)
- Nonidet P-40 (NP-40)
- Dithiothreitol (DTT)
- Phenyl fluoride methane sulfonyl (PMSF)

1.3 Apparatus

- High-speed cold centrifuge(HITACHI CF15RXII, etc.)
- Vortex mixer

2. Method

2.1 Preparation for reagent solutions

1) Buffer A: 10 mM HEPES-NaOH (pH7.9), 10 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5 mM PMSF
2) Buffer B: 20 mM HEPES-NaOH (pH7.9), 0.42 M NaCl, 1 mM EDTA, 1 mM DTT, 0.5 mM PMSF
3) Buffer C: 20 mM HEPES-NaOH (pH7.9), 100 mM KCl, 1 mM $MgCl_2$, 0.2 mM $CaCl_2$, 0.2 mM EDTA, 10%(v/v) glycerol, 1 mM DTT, 0.2 mM PMSF

2.2 Procedures

2.2.1 Fractionation

For cells in suspension:

1) Collect cells in an appropriate centrifuge tube, and centrifuge at 500 ×g for 10 min at 4 °C. and remove the supernatant.
2) Suspend cells in PBS(-) and transfer to an appropriate centrifuge tube.
3) Centrifuge at 2,000 rpm for 5 min at 4 °C and remove the supernatant.

For adherent cells:

1) Wash cells with PBS(-), once or two times.
2) Add PBS(-), collect cells by scraper, and transfer to an appropriate centrifuge tube.
3) Centrifuge at 1,000 rpm for 5 min at 15 °C and remove the supernatant.

2.2.2 Lysis of whole cells

4) Wash with five packed cell volumes(5PCV) of PBS(-).
5) Centrifuge at 1,500 ×g for 5 min at 4 °C, and remove the supernatant.
6) Repeat the above Step 4) to 5) two more times. (Wash the cells with buffer three times in total.)
7) Suspend by pipetting in 2PCV of Buffer A.
8) Stand on ice for 15 min.
9) Add the surfactant to the precipitate so that the final concentration is as follows, and suspend by pipetting.

   Types of surfactants:

   a) 1 %-3 % Octylglucoside
   b) 1 % CHAPS
   c) 1 % NP-40
   d) 1 % Tween20
   e) 1 % Triton X-100

10) Vortex for 10 sec.
11) Centrifuge at 20,000 ×g for 5 min at 4 °C, and transfer the supernatant(cytoplasmic extract)

and the pellet (nuclei) to each flesh tubes.

2.2.3 Separation of cytoplasmic fraction and cell membrane fraction

12) Octylglucoside is removed by dialysis. Other surfactants are diluted with Buffer C to a surfactant concentration of 0.1 %.
(Determine protein concentration by Bradford protein assay.)

13) Freeze with liquid nitrogen and store at -80 °C. [Mixture of cytoplasmic fraction and membrane fraction]

2.2.4 Preparation of nuclear extract

14) Suspend the pellet form Step 11) in 1PCV of Buffer B, and incubate for 15 min at 4 °C.

15) Centrifuge at 20,000 ×g for 5 min at 4 °C, and transfer the supernatant to a flesh tube.

16) Dialyze against 50 volumes of Buffer C at 4 °C.

17) Centrifuge at 20,000 ×g for 30 min at 4 °C, and transfer the supernatant to a flesh tube.
(Determine protein concentration by Bradford protein assay.)

18) Freeze with liquid nitrogen and store at -80 °C. [Nuclear extract]

3. Supplements

・ If the detergent concentration is 1 % or higher, it may interfere with the binding of the ligand to the target protein during affinity purification. Reduce the surfactant concentration to 0.1 % before use.