Cell-free synthesis of membrane proteins

Materials

Preparation of mRNA

1. 5x transcription buffer (CellFree Science, Matsuyama, Japan)
2. 25 mM NTP mixture (CellFree Science, Matsuyama, Japan)
3. Ribonuclease inhibitor (80 units/µl) (CellFree Science, Matsuyama, Japan)
4. SP6 RNA polymerase (80 units/µl) (CellFree Science, Matsuyama, Japan)

Wheat germ cell-free protein synthesis

1. Wheat germ extract (WEPRO1240, 240 OD) (CellFree Science, Matsuyama, Japan)
2. Creatine kinase (Roche Diagnostics, Tokyo, Japan)
3. SUB-A-MIX (CellFree Science, Matsuyama, Japan)
4. 96-well plate (TPP Techno Plastic Products, Schaffhausen, Switzerland)
5. Plate seal (AM-111) (Tokyo Garasu Kikai, Tokyo, Japan)
6. Dialysis cup (12,000 MWCO) (Cosmo Bio, Tokyo, Japan)
7. Receptacle tube (Maruemu yohki No. 2) (Maruemu, Osaka, Japan)
8. Parafilm (Bemis Flexible Packaging, Neenah, WI)

Methods

Bilayer method

Preparation of mRNA

1. Prepare 25 µl of transcription mixture as follows: 5 µl of 5x transcription buffer, 3 µl of 25 mM NTP mixture, 2.5 µl of template plasmid (1 µg/µl), 0.25 µl of Ribonuclease inhibitor, 0.3 µl of SP6 RNA polymerase, and 13.95 µl of Milli-Q water.
2. Incubate at 37℃ for 3-6 h.

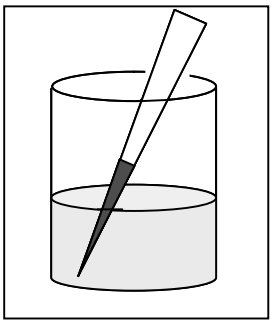
Wheat germ cell-free protein synthesis

1. Prepare 25 µl of translation mixture on ice as follows: 6.25 µl of wheat germ extract, 0.5 µl of creatine kinase (20 mg/ml Milli-Q water), 4.7 µl of 4x SUB-A-MIX, 7.5 µl

of mRNA, 2.5 µl of liposomes, and 3.55 µl of Milli-Q water.

2. Prepare 125 µl of substrate mixture on ice as follows: 31.25 µl of 4x SUB-A-MIX and 93.75 µl of Milli-Q water.
3. Transfer the 125 µl of substrate mixture to a microtiter plate.
4. Carefully pipette the 25 µl translation mixture underneath the substrate mixture to form bilayer (Figure 1).
5. Seal the plate to avoid evaporation.
6. Incubate the plate at 16˚C or 26˚C for 16-20 h. The typical yield of bi-layer-mode translation is 1-5 µg/150 µl reaction mixture.

Figure 1



Dialysis method

Preparation of mRNA

3. Prepare 25 µl of transcription mixture as follows: 5 µl of 5x transcription buffer, 3 µl of 25 mM NTP mixture, 2.5 µl of template plasmid (1 µg/µl), 0.25 µl of Ribonuclease inhibitor, 0.3 µl of SP6 RNA polymerase, and 13.95 µl of Milli-Q water.
4. Incubate at 37℃ for 3-6 h.
5. Centrifuge the transcription mixture at 20,000×*g* for 1 min.
6. Transfer the supernatant to new centrifuge tube.
7. To the supernatant, add 3.7 µl of 7.5 M ammonium acetate and 62.5 µl of ethanol. Mix well and incubate on ice for 15 min.
8. Centrifuge the mixture at 20,000×*g* for 20 min at 4˚C.
9. Discard the supernatant, and rinse the pellet with 500 µl of 70% ethanol.
10. Dry the pellet and dissolve the dried pellet in 12.5 µl of Milli-Q water.

Wheat germ cell-free protein synthesis

1. Prepare 50 µl of translation mixture on ice as follows: 12.5 µl of wheat germ extract, 1 µl of 20 mg/ml creatine kinase, 9.4 µl of 4× SUB-A-MIX, 12.5 µl of mRNA, 5 µl of liposomes, and 9.6 µl of Milli-Q water.
2. Prepare 800 µl of substrate mixture on ice according as follows: 200 µl of 4× SUB-A-MIX and 600 µl of Milli-Q water.
3. Transfer the 50 µl translation mixture into a dialysis cup.
4. Transfer 800 µl of the substrate mixture into a receptacle tube.

5. Place the dialysis cup containing the translation mixture into the receptacle tube.
6. Seal the connected portion with parafilm to avoid evaporation.
7. Incubate the reaction mixture at 16˚C or 26˚C for 16-20 h. The typical yield of dialysis method is 5-20 μg/50 μl translation mixture.

Bilayer-dialysis method

Preparation of mRNA

1. Prepare 25 μl of transcription mixture as follows: 5 μl of 5x transcription buffer, 3 μl of 25 mM NTP mixture, 2.5 μl of template plasmid (1 μg/μl), 0.25 μl of Ribonuclease inhibitor, 0.3 μl of SP6 RNA polymerase, and 13.95 μl of Milli-Q water.
2. Incubate at 37℃ for 3-6 h.
3. Centrifuge the transcription mixture at 20,000×*g* for 1 min.
4. Transfer the supernatant to new centrifuge tube.
5. To the supernatant, add 3.7 μl of 7.5 M ammonium acetate and 62.5 μl of ethanol. Mix well and incubate on ice for 15 min.
6. Centrifuge the mixture at 20,000×*g* for 20 min at 4˚C.
7. Discard the supernatant, and rinse the pellet with 500 μl of 70% ethanol.
8. Dry the pellet and dissolve the dried pellet in 12.5 μl of Milli-Q water.

Wheat germ cell-free protein synthesis

1. Prepare 50 μl of translation mixture on ice as follows: 12.5 μl of wheat germ extract, 1 μl of 20 mg/ml creatine kinase, 9.4 μl of 4× SUB-A-MIX, 12.5 μl of mRNA, 5 μl of liposomes, and 9.6 μl of Milli-Q water.
2. Prepare 1,200 μl of substrate mixture on ice according as follows: 300 μl of 4× SUB-A-MIX and 900 μl of Milli-Q water.
3. Transfer the 150 μl the substrate mixture into a dialysis cup.
4. Transfer the 50 μl translation mixture underneath the substrate mixture in the dialysis cup to form bilayer.
5. Transfer 800 μl of the substrate mixture into a receptacle tube.
6. Place the dialysis cup into the receptacle tube.
7. Seal the connected portion with parafilm to avoid evaporation.
8. Incubate the reaction mixture at 16˚C or 26˚C for 16-20 h.