



ENDEXT[®] Technology

Translation by using bilayer method

1 Materials

Item	Concentration
WEPRO®1240	240 OD/ml
Creatine kinase	20 mg/ml or 1 mg/ml (*1)
SUB-AMIX®	1 x

Note(*1): The concentration of the starting creatine kinase solution is 20 mg/ml. Use it as it is for preparing the translation mixture for large scale translation. For small scale translation (see Section 2.1), dilute it with nuclease-free water to 1 mg/ml. In both cases, the final concentration of creatine kinase in translation mixture should be 40 ng/μl.

2 Protocol

- 2.1** Cool the mRNA tube down to the room temperature. Thaw WEPRO®1240 under running water, and immediately after thawing, place it on ice. Thaw creatine kinase on ice. Before adding mRNA into the translation mixture, resuspend the white pellet (magnesium pyrophosphate) in the tube by pipetting gently. Prepare translation mixture on ice according to the mixing formula shown below and mix gently by pipetting. Avoid bubbling.

Reagents	Working vol. (μl)		Final conc.
	Small scale (*1)	Large scale (*2)	
mRNA	10	250	1/2 vol.
WEPRO®1240 (240 OD/ml)	10	250	120 OD/ml
Creatine kinase (1 mg/ml)	0.8	-	40 ng/μl
Creatine kinase (20 mg/ml)	-	1	40 ng/μl
Total	20.8	501	

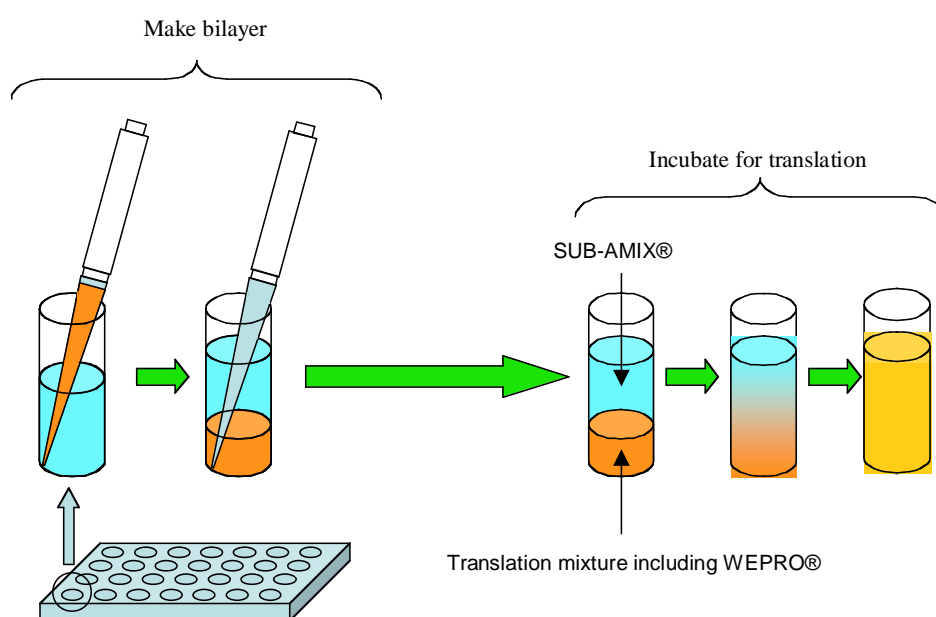
Note(*1): For small scale protein expression using a standard 96 multi-well plate. It is for a translation reaction volume of 226.8 μ l per well.

Note(*2): For large scale protein expression using a standard 6 multi-well plate. It is for a translation reaction volume of 6 ml per well.

2.2 Thaw 1x SUB-AMIX® on ice and mix gently by pipetting. Pipet out 1x SUB-AMIX® (*1) and add it into a well of a flat bottom plate.

Note(*1): The volume of 1x SUB-AMIX® is 206 μ l for small scale, 5.5 ml for large scale.

2.3 Carry out bilayer reaction: Carefully transfer the translation mixture into the bottom of the well containing SUB-AMIX® to form bilayer with the translation mixture in the lower layer and SUB-AMIX® in the upper layer as illustrated below. (Important !!)



- 2.4 Seal the well with paraffin film to avoid evaporation.
- 2.5 Incubate at 26°C for 8 to 16 hours. Higher activity, solubility, and productivity are expected at lower temperatures (ex. 15°C for 20 hours).