



ENDEXT[®] Technology

Repeat-batch method

Ver. 1

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General Remarks

This protocol is written for 1-ml translation reaction. It is applicable up to 5 ml of translation reaction.

1 Transcription

(1) Prepare a transcription solution of the following compositions:

	1 sample (µl)	Final conc.
Nuclease-free water	188.6	-
5x Transcription buffer	80	1x
1 M Mg(CH ₃ COO) ₂	6.4	16 mM
25 mM NTP	80	5 mM
Plasmid (1 µg/µl)	40	0.1 µg/µl
RNase inhibitor (80 U/µl)	2.5	0.5 U/µl
SP6 Polymerase (80 U/µl)	2.5	0.5 U/µl
Total	400	

(2) Incubate at 37 °C for 6 hours.

(3) Centrifuge at 4,000xg at 26 °C for 20 minutes to remove white precipitate, which is magnesium phosphate.

(4) Collect the supernatant.

Note: If the white precipitate is not removed, insoluble precipitate will appear when the transcription solution is mixed (p. 3, Section 2) with WEPRO®.

(5) Use 0.3 ml of the supernatant for 1 ml of subsequent translation reaction.

2 Translation

2-1 Preparation of translation solution

(1) Prepare a translation solution of the following compositions:

	1 sample (µl)	Final conc.
mRNA	300	-
1x SUB-AMIX	420	
WEPRO [®] (240 OD/ml)	250	60 OD/ml
Creatine kinase (20 µg/µl)	30	0.6 µg/µl
Total	1000	

(2) Add an equal volume (1 ml in this case) of 1x SUB-AMIX[®] and mix by pipetting gently.

(3) Transfer the whole volume to Amicon Ultra-15 10 k MWCO (Millipore, Catalogue No. UFC9-010 series).

(4) Centrifuge (possibly in multiple steps) to bring down the volume to the initial volume (1 ml). For initial volumes more than 1 ml, centrifugal gravity is reduced to protect the filter of Amicon Ultra-15 against breakage (Table 1).

Table 1 Relation between volume of the translation solution and centrifugal gravity

Initial volume of the translation	1 ml	2 ml	3 ml	4 ml	5 ml
Additional vol. of SUB-AMIX [®]	1 ml	2 ml	3 ml	4 ml	5 ml
Centrifugation gravity	4,000xg	4,000xg	3,000xg	3,000xg	2,000xg
Time	8 min	10 min	18 min	22 min	38 min

Notes: If centrifuge brings the solution volume too far down, add 1x SUB-AMIX[®] to bring it back to the initial volume.

(5) Repeat the steps (2) and (4) 3 times.

2-2 Repeat-batch translation reaction

- (1) Incubate the above solution at 26 °C for 30 minutes.
- (2) Add an equal volume (1 ml in this case) of the 1x SUB-AMIX[®] and mix by pipetting gently.
- (3) Centrifuge (possibly in multiple steps) to bring down the solution volume to the initial volume. Refer to Table 1 on page 3 regarding the centrifugal gravity for various initial volumes.

Notes: If centrifuge brings the solution volume too far down, add 1x SUB-AMIX[®] to bring it back to the initial volume.

- (4) Mix by pipetting gently.
- (5) Repeat steps (2) through (4) 10 times.

Note: It may be possible to increase the protein synthesis yield by increasing incubation time.