ENDEXT® Technology

Instruction for Reagents

1 WEPRO®

Store at a temperature below -80 degrees C.
WEPRO®(the wheat germ extract) is sensitive to the vibration in a liquid phase. After melting WEPRO® with flowing water, place immediately on ice. Do not expose reagent to 3 freeze-thaw cycles.

Upon Thawing WEPRO® for the first time, keep the remaining WEPRO in separate containers for the next use. When re-freezing, freezing with liquid nitrogen is recommended. After the 3rd cycle of freeze-thaw, it is possible that protein synthesis activity falls depending on the method of handling.

2 SUB-AMIX®

This product consists of a set of 4 reagents(S1, S2, S3, S4) in 40 x concentration. Store all 4 reagents, S1, S2, S3, S4 at a temperature below -20 degrees C. 4 reagents, S1, S2, S3, S4 are observed to show no change in reaction efficiency after 10 freeze-thaw cycles. For convenience, subdivide the concentrated reagents into 1ml and store below -20 degrees C.

To prepare 2x SUB-AMIX®, add 1ml each of S1 through S4 to 15ml of RNase-free water as agitating to make 20ml solution. Using the 2x SUB-AMIX® solution, add equal volume of RNase-free water to make 1x SUB-AMIX® solution. When 4 reagents are mixed first, **precipitation** may occur. In this case it takes time to dissolve precipitates.

Remaining 1x and 2x SUB-AMIX® should be stored at -80 degrees C. Do not expose 1x and 2x mixture to multiple freeze-thaw cycles. Depending on the method of handling, the reaction efficiency can decline.

2.1 Composition of 1 x SUB-AMIX®

Composition	Concentration
L-Ala	0.3 mM
L-Arg	0.3 mM
L-Asn	0.3 mM
L-Asp	0.3 mM
L-Cys	0.3 mM
L-Gln	0.3 mM
L-Glu	0.3 mM
Gly	0.3 mM
L-His	0.3 mM
L-Ile	0.3 mM
L-Leu	0.3 mM
L-Lys	0.3 mM
L-Met	0.3 mM
L-Phe	0.3 mM
L-Pro	0.3 mM
L-Ser	0.3 mM
L-Thr	0.3 mM
L-Trp	0.3 mM
L-Tyr	0.3 mM
L-Val	0.3 mM
Potassium Acetate	100 mM
Magnesium Acetate	2.7 mM
Disodium Creatine Phosphate Tetrahydrate	16 mM
Spermidine	0.4 mM
ATP	1.2 mM
GTP	0.25 mM
Dithiothreitol	4 mM
HEPES-KOH (pH 7.8)	30 mM

3 5x TB(Transcription Buffer)

After freeze-thaw, subdivide 5xTB convenient for your use.
For storage, keep it at a temperature below -20 degrees C. It is confirmed that freeze-thaw for this product can be repeated up to 10 freeze-thaw cycles.

4 25mM NTP mix

ATP, GTP, CTP, UTP in this NTPmix are prepared at the final concentration of 25mM. For storage, keep it at a temperature below -20 degrees C. It is confirmed that freeze-thaw for this product can be repeated up to 10 times.

5 SP6 RNA Polymerase, RNase Inhibitor

Please see the instruction attached on to the products. For storage, keep it at a temperature below -20 degrees C.

6 Creatine Kinase

When you use WEPRO® 1240, CREATINE KINASE has to be added into the reaction mixture. Please follow the preparation below.

6.1 Dissolve creatine kinase with RNase free water to 20 µg/µl concentration. For storage, subdivide the creatine kinase solution and keep it at -80 degrees C. Avoid multiple freeze-thaw process, otherwise, the effectiveness of creatine kinase may be decreased. The final concentration of creatine kinase in reaction mixture should be 40 ng/µl.

Example) Reaction Mixture Preparation for Manual Method

	1 sample	final conc.
WEPRO® 1240 (240 OD /ml)	250 µl	120 OD /ml
Creatine kinase (20 µg /µl)	1 µl	40 ng /µl
mRNA	250 µl	
total	501 µl	

Note: Creatine Kinase(CK) Roche CatNo.127566

Note: In the case of small scale (total 20.8 µl), Creatine kinase (20 µg /µl) should be diluted to 1 µg /µl.