ENDEXT® Technology

Transcription of plasmid DNA

Ver.1.3 Mar 31, 2006

1 Materials

Item	Concentration
Transcription buffer	5 x
NTP mix	25 mM
RNase inhibitor	80 U/µl
SP6 RNA Polymerase	80 U/µl
pEU vector (*1)	1 µg/µl

Note(*1): pEU vector is a plasmid DNA specifically designed for wheat germ cell-free protein synthesis system.

2 Protocol for bilayer method

2.1 Thaw 5x transcription buffer and 25 mM NTP mix on ice. Place and keep all reagents on ice during handling. Prepare transcription mixture on ice according to the mixing formula shown below and mix gently by pipetting.

Reagents	Working vol. (µl)		Final conc.
	Small scale (*2)	Large scale (*3)	
5 x Transcription buffer	4	50	1 x
25 mM NTP mix	2	25	2.5 mM
RNase Inhibitor (80 U/µl)	0.25	3.125	1 U/µl
SP6 RNA Polymerase (80 U/µl)	0.25	3.125	1 U/µl
pEU vector (*1) (1 µg/µl)	2	25	100 ng/µl
Nuclease free water	11.5	143.75	
Total	20	250	

Note(*1): pEU vector is a plasmid DNA specifically designed for wheat germ cell-free protein synthesis system.

Note(*2): For small scale protein expression using a standard 96 multi-well plate. It is for a translation reaction volume of 226.8 µl per well.

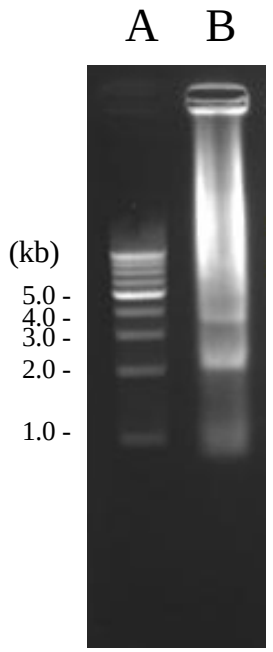
Note(*3): For large scale protein expression using a standard 6 multi-well plate. It is for a translation reaction volume of 6 ml per well.

2.2 Incubate at 37°C for 6 hours in a thermal cycler or incubator.

Note: White pellet that appears during incubation is magnesium pyrophosphate.

2.3 After incubation, confirm the mRNA quality by the ordinary method of agarose gel electrophoresis.

Note: A smear or ladder pattern, especially that of mRNA of a small molecular weight (50-500 bases), indicates possible degradation of mRNA probably caused by RNase. In that case, repeat the preparation of plasmid DNA. An example of mRNA produced in high quality is shown below:



A: Molecular weight marker (DNA)
B: mRNA
*1% agarose gel