ENDEXT® Technology

Transcription from PCR product

Ver. 1

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1 Materials

Item	Concentration
Transcription buffer	5 x
NTP mix	25 mM
RNase inhibitor	80 U/μl
SP6 RNA Polymerase	80 U/μl
2nd PCR product	0.5 μg/μl

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 (*1)	Large scale (*2)	
5 x Transcription buffer	6	75	**1.5 x**
25 mM NTP mix	3	37.5	3.75 mM
RNase Inhibitor (80 U/μl)	0.3	3.75	1.2 U/μl
SP6 RNA Polymerase (80 U/μl)	0.6	7.5	2.4 U/μl
2nd PCR product (0.25 μg/μl)	4	50	50 ng/μl
Nuclease-free water	6.1	76.25	
Total	20	250	

Note(*1): 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(*2): For large scale protein expression using a standard 6 multi-well plate. It is for translation reaction volume of 6 ml per well.

2.2 Incubate at 37°C for 4-6 hours.

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.