2-steps RT-PCR Kit

Product Code: RTPL26

Description:

The 2-step RT-PCR kit is specially designed to provide reliable synthesis of full-length cDNA and convenient application of cDNA in PCR. M-MuLV RNase H^- synthesizes complementary DNA strand initiating from a specific primer, oligo d(T) or random hexamer. The absence of RNase H enhances the synthesis of long cDNA as the RNA strand does not degrade in DNA-RNA hybrid during first strand cDNA synthesis. With the variety of kit options for standard PCR and long PCR, the 2-step RT-PCR kit provides flexibility in an easy use format.

Features:

- High yield: Absence of RNase H activity allows high amount of full length cDNA synthesis with RNA templates up to 10kb.
- Flexible: Wide selection of primers, oligo d(T) or random hexamer. *Taq* DNA polymerase and Max*Taq* DNA polymerase for amplification of short and long DNA fragment.
- Highly compatible with various routine PCR amplifications.
- Allows synthesis of full length cDNA from various RNA templates up to 9.8kb.
- High capacity and able to copy up to 2μg of purified mRNA

Quality Control:
All preparations are assayed for contaminating endonuclease, exonuclease and non-specific RNase activities.

Kit Components:	**Product**	**Sample**
Reverse Transcription		
M-MuLV Reverse Transcriptase	10,000u	1000u
10X Buffer M-MuLV	0.25ml	25μl
10mM dNTPs mix	0.25ml	25μl
Oligo $d(T)_{18}$ (40μM)	100μl	10μl
Random hexamer (50ng/μl)	100μl	10μl
Nuclease-free Water	1ml	1ml
PCR		
Chromo Max*Taq* DNA Polymerase	500u	50u
10X ViBuffer A	2ml	1ml
10X ViBuffer S	1ml	1ml
50mM $MgCl_2$	1ml	1ml
10mM dNTPs mix	0.25ml	25μl
Nuclease-free Water	2ml	1ml

Storage and Stability
Stable at -20°C for 1 year if properly stored.

M-MuLV Storage Buffer:
200mM NaCl, 10mM potassium phosphate (pH7.5), 0.1mM EDTA, 7mM β-mercaptoethanol and 50% glycerol.

10X Buffer M-MuLV:
500mM Tris-HCl (pH8.3 at 25°C), 750mM KCl, 30mM $MgCl_2$ and 100mM DTT.

Chromo Max*Taq* DNA Polymerase Storage Buffer:
20mM Tris-HCl (pH8.0 at 22°C), 100mM KCl, 0.5% Tween™20, 0.5% Nonidet-P40, 0.1mM EDTA, 1mM DTT, colour dyes and 50% glycerol.

10X ViBuffer A:
500mM KCl, 100mM Tris-HCl (pH9.2 at 20°C) and 0.1% Triton™ X-100.

10X ViBuffer S:
160mM $(NH_4)_2SO_4$, 500mM Tris-HCl ((pH9.2 at 22°C), 17.5mM $MgCl_2$ and 0.1% Triton™ X-100.

Preliminary Considerations:

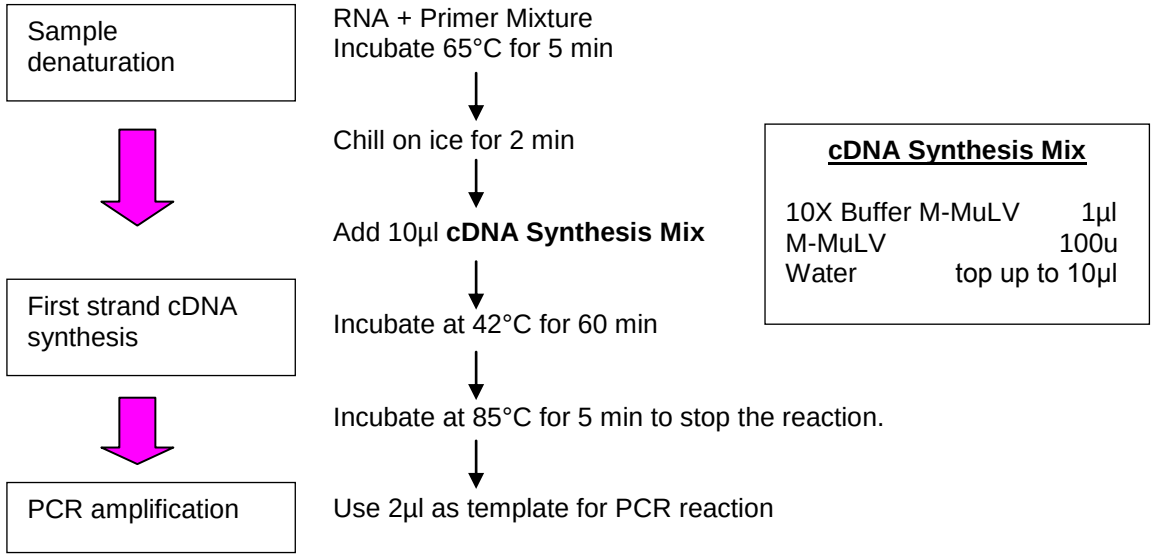
Primers

Gene-specific primers, oligo d(T) and random hexamer can be used in reverse transcription. Gene-specific primers transcribe only specific gene sequences and therefore increase the specificity. Oligo d(T) transcribes all poly(A)+ mRNA, includes eukaryotic mRNA and viruses with poly(A) tail. Random hexamer transcribes all RNA from transcript. The use of random hexamer is not recommended when total RNA is used as template, as rRNA and tRNA may also be primed and copied, resulting in a lower efficiency of cDNA transcribed from mRNA.

RNA templates

Quality and quantity of RNA templates determine the efficiency of reverse transcription process. The presence of minute amount of RNases can degrade the RNA and affect the cDNA length transcribed. To prevent RNases contamination, RNA purification have to be carried out in a RNase-free environment. Glassware, plasticware and reagents should be essentially RNase-free.

Reaction Setup



Recommended protocol for first strand cDNA synthesis

1. Mix the reagents well and centrifuge the tubes briefly before pipetting.
2. Prepare the RNA-primer mixture as below in a 0.2ml microcentrifuge tube.

Component	**Amount/Volume**
Template: total RNA or poly A(+) mRNA	1 – 10µg (Recommended 10µg) 0.01 – 2µg (Recommended 1µg)
Primer: oligo $d(T)_{18}$ (40µM) or random Hexamers (50ng/µl) or gene-specific primer	1µl 1µl Volume depends on the primer stock concentration
10mM dNTPs mix	1µl
Nuclease-free water	Top up to10µl

3. Incubate the mixture at 65°C for 5 minutes and chill on ice for 2 minutes.
4. Briefly spin down the mixture.
5. Prepare the following cDNA Synthesis Mix in the order indicated:

Component	**Volume**
10X Buffer M-MuLV	2µl
M-MuLV Reverse Transcriptase	100 unit
Nuclease-free Water	Top up to 10µl

6. Add 10µl of the cDNA Synthesis Mix into each RNA-primer mixture. Mix gently and centrifuge briefly.
7. Incubate at 42°C for 60 min.
   Note:If random hexamer is used, incubation at 25°C for 10 min is needed prior to incubation at 42°C to prevent to dissociation of primer from template while the temperature increases. If gene specific primer or oligo d(T) is used, this step can be ignored.
8. Terminate the reaction by incubate the tubes at 85°C for 5 min. Chill the tubes on ice and collect the solution by centrifuge the tube briefly.
9. The synthesized cDNA can be directly used in PCR, by addition of 1 – 2µl of the cDNA reaction mixture to a 25µl PCR reaction.

Amplification of cDNA template

1-5µl of cDNA can be added directly in PCR reaction mixture. Amount of cDNA to be added is recommended to be not more than 10% of the total volume of PCR reaction.

Suggested Initial PCR Condition for various PCR product sizes:

Reagents \ Product size	100bp-5kb	5-8kb	>8kb
dNTPs mix	0.1mM	0.2mM	0.3mM
1X Vi Buffer	A	A	S
Ultrapure DMSO or formamide	-	3%	3%
DNA polymerase	Refer to Table below		

Recommended units For DNA Polymerases per 50µl reaction volume:

Product sizes \ DNA Polymerase	*Taq* / Chromo *Taq*	Max*Taq* / Chromo Max*Taq*
0.1-5kb	2.0	2.0
5-8kb	2.5	2.0
>8kb	2.5	2.0

Thermocycler set up for different PCR product sizes:

Product size	0.1-5kb	5-8kb	>8kb
Pre-denaturation	94°C, 2 min	94°C, 2 min	94°C, 2 min
Denaturation	94°C, 30 sec	94°C, 12 sec	94°C, 12 sec
Annealing*	50-68°C, 30 sec	50-68°C, 30 sec	50-68°C, 30 sec
Extension per kb	72°C, 30 sec	72°C, 45 sec	68°C, 1 min
Cycles	25-35	25-35	25-35
Final Extension	72°C, 7 min	72°C, 7 min	72°C, 7 min

*Primer dependent

Troubleshooting

Problem	Possibility	Suggestion
Low yield or no amplification product	Concentration of template RNA is too low	Increase the amount of template RNA. Use 5µg for total RNA or 1µg for mRNA.
	Poor quality RNA	Check the purity and integrity of the purified RNA. Use commercial RNA extraction kit.
	RNase contamination	Always wear gloves and work in a RNase-free environment.
	Secondary structure of RNA blocks cDNA synthesis	Reaction temperature can be optimized between 37-48°C. Make sure the RNA pre-denaturation step was carried out. Try another priming method.
	Missing component in the reaction mixture	Check the reaction set up and components. Repeat the reaction.
	PCR reaction not optimal	Optimize annealing temperature and increase number of cycle.