First Strand cDNA Synthesis Kit

Sample Kit for 10Rxn

Description:

Kit includes The M-MLV Reverse Transcriptase RNase H-. Enzyme is a genetically modified M-MLV Reverse Transcriptase which exhibits RNA or DNA dependent DNA polymerase, but lacks ribonuclease H activity. This enzyme can synthesize a complementary DNA strand initiating from a primer using RNA or DNA templates. Removal of the RNase H activity results in an increase of full-length cDNA products.

Application:

- cDNA synthesis
- RNA analysis by primer extension
- DNA labeling

Reagents Provided:

- M-MLV Reverse Transcriptase RNase H- 2000U
- 5x RT Reaction buffer 1 (with MgCl2 and DTT) 0.25 M Tris-HCl, 0.5 M KCl, 30 mM MgCl2, 25 mM DTT
- 5x RT Reaction buffer 2 (Mg2+ and DTT free) 0.25 M Tris-HCl, 0.5 M KCl
- 25 mM MgCl2 • 20 mM MnCl2
- Oligo (dt) 50µM 15µL
- Random Hexamer 50µM 15µL
- dNTP Mix 20mM 15µL
- RNase Inhibitor 8µl 5mg/mL

Recommended cDNA synthesis reaction mix: for 5x RT Reaction buffer 1 (with MgCl2 and DTT)

Components	Volume	Final conc.
M-MLV Reverse Transcriptase RNase H- (200 U/µl)	1 µl	4 U/µl
5x RT Reaction buffer 1	10 µl	1 x
20 mM dNTP mix	0.5-1.25 µl	200-500 µM
100 mM DTT	0-2.5 µl	5-10 mM
RNase inhibitor *		optional
Oligo(dT) / random primer or gene-specific primer per µg of RNA		250-500 ng / 50-100 ng
RNA	2-5 µg	
H_2O	Up to 50 µl	

- **Recommended cDNA synthesis reaction mixes:** for 5 x RT Reaction buffer 2 (Mg2+ and DTT free)

Components	Volume	Final conc.
M-MLV Reverse Transcriptase RNase H- (200 U/µl)	1 µl	4 U/µl
5x RT Reaction buffer 2	10 µl	1 x
20 mM $MnCl_2$	5 µl	2 mM
100 mM DTT	2.5-5 µl	5-10 mM
20 mM dNTP mix	0.5-1.25 µl	200-500 µM
RNase inhibitor *		optional
Oligo(dT)/ random primer or gene-specific primer per µg of RNA		250-500 ng / 50-100 ng
RNA	2-5 µg	
H_2O	Up to 50 µl	

Components	Volume	Final conc.
M-MLV Reverse Transcriptase RNase H- (200 U/µl)	1 µl	4 U/µl
5x RT Reaction buffer 2	10 µl	1 x
25 mM $MgCl_2$	6-12 µl	3-6 mM
100 mM DTT	2.5-5 µl	5-10 mM
20 mM dNTP mix	0.5-1.25 µl	200-500 µM
RNase inhibitor *		optional
Oligo(dT)/ random primer or gene-specific primer per µg of RNA		250-500 ng / 50-100 ng
RNA	2-5 µg	
H_2O	Up to 50 µl	

Protocol: Reaction volume 50 µl

1. In a sterile microcentrifuge tube, add RNA and primer(s) in a total volume of 15 µl water
2. Heat the tube at 700C for 5-10 minutes, then 10-15 minutes at room temperature (for specific primer) or place in ice in case of Oligo(dT) or random primers
3. Spin for a few seconds
4. Add water
5. Add dNTPs
6. Add 5 x RT buffer 1 or 5x RT Reaction buffer 2 and DTT and MnCl2 or 5x RT Reaction buffer 2 and DTT and MgCl2
7. Add RNase inhibitor (optional)
8. Add M-MLV Reverse Transcriptase RNase H-
9. Mix gently and incubate at 370C for 30-90 minute