vivantis

Product Datasheet

Viva cDNA Synthesis Kit

Product No : cDSK01-100
Quantity : 100 reactions

Lot :
Expiry Date :

Store at -20°C

info@vivantechnologies.com

Description:
Viva cDNA Synthesis Kit is specially designed to provide reliable synthesis of full-length cDNA. 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. This cDNA synthesis kit is readily compatible with various cDNA-dependent downstream applications.

Features:
- Absence of RNase H activity allows high yield of full length cDNA synthesis with RNA templates up to 10kb
- Wide selection of primers, oligo d(T) or random hexamer
- Highly compatible with various downstream applications
- Allows synthesis of full length cDNA from various RNA templates
- High capacity and able to copy up to 2µg of purified mRNA

Applications:
- Traditional PCR amplification or real-time PCR quantification
- Storage of selective and functional genetic information by removing of introns
- Cloning of the gene of interested enables horizontal gene transfer (HGT)
- cDNA probe library construction with large and full-length inserts
- cDNA microarray for gene discovery and clinical diagnosis via gene pattern screening
- mRNA expression studies and storage of proteins material at genomics level

Quality Control:
All preparation is assayed for contaminating endonucleases, exonucleases and non-specific RNase activities.

Kit Components	Product
M-MuLV Reverse Transcriptase	10,000u
10X Buffer M-MuLV	0.25ml
10mM dNTPs mix	0.25ml
Oligo d(T)$_{18}$ (40µm)	100µl
Random hexamer (50ng/µl)	100µl
Nuclease-free water	1ml x 2

Storage and stability
Stable at -20°C for 18 months if reagents are properly stored.

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 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 RNase contamination, RNA purification has to be carried out in a RNase-free environment. Glassware, plasticware and reagents should be essentially RNase-free.

Recommended protocol for first strand cDNA synthesis
1. Mix the reagents well and centrifuge the tubes briefly.
2. Prepare the RNA-primer mixture as below:

Component	Amount/Volume
Template: total RNA **or** poly(A)+ mRNA)	1 - 10µg (recommend 10µg) 0.01 - 2µg (recommend 1µg)
Primer: Oligo d(T)$_{18}$ **or** Random hexamers **or** Gene-specific primer	1µl 1µl Depends on stock conc.
10mM dNTPs mix	1µl
Nuclease-free water	Top up to 10µl

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

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

5. Add 10µl of the cDNA Synthesis Mix into each RNA-primer mixture. Mix gently and centrifuge briefly.
6. Incubate at 42°C for 60 minutes.
7. Terminate the reaction by incubate the tubes at 85°C for 5 min. Chill the tubes on ice and centrifuge the tube briefly.
8. The synthesized cDNA can be directly used for downstream application or stored at -20°C.

Troubleshooting
1. Too little or no cDNA product

Too little RNA template	• Used purified mRNA instead of total RNA template. • Check RNA quality, concentration and degradation. • Increase the amount of starting RNA template.
RNase contamination possibility	• Add control RNA into sample
Too high enzyme amount	• Use 10 unit or less enzyme unit for 1µg RNA template in a 20µl cDNA reaction.

2. False positive signal on negative control

RNA contamination	• Re-prepare all reagents. • Ensure working bench is clean and RNA-free.

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