cDNA Master Mix, 2X

REF RT5202 | 50 TESTS

Wet or Dry Ice | RUO | **Store at: -20°C**

Components

Kit Contents	Quantity/ Volume
cDNA Master Mix, 2X	500µl

Description

Sinaclon cDNA Master Mix is specially designed to provide synthesis of full-length cDNA from mRNA or total RNA templates. The advantage of this master is the simultaneous use of both oligo$(dT)_{18}$ primers and random hexamer in the buffer, which increases the efficiency of the master, reduces pipetting steps, and reduces user errors. M-MulV RNase –H synthesizes complementary DNA strand initiating from oligo$(dT)_{18}$ 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. The RNase inhibitor, supplied in the RT Enzyme Mix, effectively protects RNA from degradation at temperatures up to 50˚C.

Storage and Stability

Stable at -20°C for 2 years if properly stored.

Preliminary Considerations Primers

Oligo$(dT)_{18}$ transcribes all poly (A) + mRNA, includes eukaryotic mRNA and viruses with poly(A) tail. Random hexamer initiates cDNA synthesis from the total RNA population (rRNA and mRNA).

RNA templates

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

Protocol

Recommended protocol for first strand cDNA synthesis

1. After thawing, mix and briefly centrifuge the components, Store on ice.
2. Prepare the mixture as below in a sterile, nuclease-free tube on ice.

component	Volume/ Concentration
Template: total RNA	Up to 1µg
cDNA Master Mix	10µl
DEPC-treated water	Top up to 20µl

3. Briefly spin down the mixture.
4. Incubate at 55°C for 30-60min.
5. Terminate the reaction by incubate the tubes at 95°C for 10min. Chill the tubes on ice and collect the solution by centrifuge the tube briefly.
6. The synthesized cDNA can be directly used in PCR, by addition of 2-5µl of the cDNA reaction mixture to a 25/50µl PCR reaction.

Quality Control

RT-PCR using 50ng of control GAPDH RNA and GAPDH control primers generated a visible product on agarose gel after DNA Safe Stain staining.

Signs

Signs	Definitions
	Product shipping conditions
RUO	For Research Use Only
	Number of usable tests
	Name and address of the manufacturer of the product
REF	Product technical code

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