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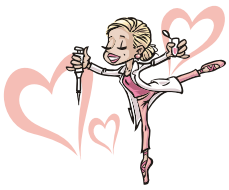
roboklon

1-STEP RT-PCR KIT

- for one-step RT-PCR assays -



Reverse Transcriptase
"Hot Start" DNA Polymerase
RNase Inhibitor

Cat. No.	Size
E0803-01	25 reactions
E0803-02	100 reactions

Storage Conditions:
Store at -20°C

Quality Control:
All preparations are assayed for contaminating endonuclease and exonuclease and nonspecific RNase and single- and double- stranded DNase activities.

Component	25 Rxn Kit	100 Rxn Kit
Master Enzyme Mix (Reverse Transcriptase, "HotStart" DNA Poly-merase, RNase Inhibitor)	25 µl	100 µl
2x Master Buffer Mix	350 µl	2 x 700 µl
RNase free water	1.0 ml	4 x 1.0 ml

Direct RT-PCR starting from RNA. The dART OneStep RT-PCR Kit is a convenient system for the setup of single-tube, one-step RT-PCR reactions.

Description:

- The dART OneStep RT-PCR Kit contains an enzyme master mix including the highly processive dART reverse transcriptase, thermostable "Hot Start" DNA polymerase and a unique RNase Inhibitor, capable of maintaining RNase inhibition at elevated temperatures.
- The 2x concentrated buffer system included with the enzyme master mix contains dNTPs, stabilizers and reaction enhancers, providing a highly optimized reaction environment for DNA polymerase, reverse transcriptase and RNase Inhibitor.
- During the reverse transcription step at elevated temperatures, "Hot Start" DNA polymerase remains inactive. Polymerase activity is released during the initial denaturation step, resulting in sensitive, efficient and artifact-devoid PCR amplification.
- The kit is designed towards sensitive amplification DNA from any RNA with high specificity in a one-step process. This system is designed for analytic applications as well as for cloning purposes.
- Compatible with emulsion reactions such as provided with the Micellula Emulsion & DNA Purification kit (Cat. No. E3600-01).

dART One Step RT-PCR Protocol:

This reaction allows cDNA synthesis starting from small to medium RNA amounts. 10 ng up to 2 µg RNA permits synthesis of full length cDNA synthesis.

1. Prepare Reaction Mix: For each reaction, combine these components in a 0.2 ml tube:

Reaction Mix

2x Master Buffer Mix.....12.5 µl
Sense Primer [10 µM].....1 µl
Reverse (Antisense) Primer [10 µM]. .1 µl
RNA [10 ng-2 µg].....x µl
Master Enzyme Mix.....1 µl
RNase free water.....to 25 µl

Total volume: 25 µl.

2. Gently mix the reaction by pipetting. If required, spin down briefly to collect the liquid at the bottom of the tube.

3. Transfer the sample to thermal cycler. Incubate as follows: 30 min at 50°C, followed by standard PCR with annealing temperatures optimized towards primers in use.

Reverse Transcription	50°C	30 min
Initial Denaturation	94°C	5 min
PCR: Cyclic Amplification, 30 - 40 Cycles		
Denaturation	94°C	30 s
Annealing	50°C-65°C	30 s
Extension	72°C	1 min/1 kb
PCR: End of Cyclic Amplification		
Final Extension	72°C	5 min

4. Analyze 5-20 µl of RT-PCR sample by agarose gel electrophoresis with suitable molecular markers.

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