NG dART RT-PCR kit

Cat. No.	Size
E0802-01	25 reactions
E0802-02	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.

NG dART RT-PCR kit is first strand cDNA synthesis kit convenient for two step RT-PCR. Kit is based on modified reverse transcriptase with improved thermostability (up to 65°C) and processivity. Has easy to use format to save time and limit the possibility of pipeting errors.

NG dART RT-PCR kit allows to amplify DNA from any RNA with high specificity and sensitivity. NG dART RT mix contains dART reverse transcriptase and RNase Inhibitor preventing from RNases A,B and C. 5X NG cDNA buffer contains optimized for RT buffer and dNTPs.
cDNA synthesis is performed in the first step using either total RNA or poly(A)$^+$-RNA primed with oligo(dT), random hexamers primers or reverse gene specific primer. The second step, in separate tube is PCR where cDNA is a template and specific primers are used to amplify double-stranded DNA of interest using high fidelity OptiTaq DNA Polymerase.

COMPONENTS OF THE KIT

NG dART RT-PCR kit	E0802-01	E0802-02
NG dART RT mix	25 µl	100 µl
5X NG cDNA Buffer	150 µl	600 µl
Oligo(dT)$_{20}$ (50 µM)	25 µl	100 µl
Random hexamers (200 ng/µl)	25 µl	100 µl
dNTPs mix 5 mM each	50 µl	200 µl
OptiTaq DNA Polymerase 2.5 U/ µl	25 µl	100 µl
10X Pol Buffer C with $MgCl_2$	250 µl	1.0 ml
RNase-free Water	1 ml	4 x 1.0 ml

1. Houts, G.E., Masakau, M., Ellis, C., Beard, D. and Beard,J.W. (1979) J. Virol. 29, 517-522.

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First strand cDNA synthesis:

1. Place 5X NG cDNA Buffer at room temperature, thaw and vortex gently. Visible white precipitate will dissolve and clear buffer is ready for use.

2.Assambly reaction in RNAse-free tube as follows :

Component:	**Amount:**
5X NG cDNA Buffer	4 µl
primer*	1 µl
RNA (10 ng-5 µg)	x µl
NG dART RT mix	1 µl
RNase-free Water	to 20 µl

* 50 µM Oligo$(dT)_{20}$, 200 ng/µl random hexamer primer or 10 µM reverse gene specific primer.

3.Transfer the sample to preheated to appropriate temperature thermal cycler. Incubate as follows:

Oligo$(dT)_{20}$ primed:	30-60 min at 50°C (or 35-65°C)
Gene specific primed:	30-60 min at 50°C (or 35-65°C)
Random hexamer primed:	25°C for 10 min, followed by 20-50 min at 50°C (or 35-65°C).

NOTE. 50°C is suitable temperature for most targets. For G-C rich RNA templates or with complex secondary structure temperature can be increased to 65°C.

4. Terminate the reaction by incubating at 85°C for 5 min.

5. cDNA is ready for PCR, can be used immediately or stored at -20°C. Use 2-5 µl for 50 µl PCR.

PCR with OptiTaq DNA Polymerase:

The final magnesium concentration is 1.5 mM in reaction and in some cases there is a need of titration of magnesium to obtain best results.

1. Mix as follows all reagents in 0.2-0.5-ml tube.

Component:	**Amount:**
cDNA template	2-5 µl
10X Pol Buffer C	5 µl
dNTPs mix 5 mM each	2 µl
10 µM sense primer	1 µl
10 µM reverse primer	1 µl
OptiTaq DNA Polymerase 2.5 U/ µl	1 µl
RNase-free Water	to 50 µl
	50 µl

2. Mix gently by pipeting.
3. Incubate at 94°C for 3 min, then perform 20-40 cycles of PCR with optimized conditions for Your sample (1 min/kb extension time at 68-72°C).
4. Analyze 10-20 µl of PCR sample by agarose gel electrophoresis.

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