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, exonuclease, nonspecific RNase, single- and double-stranded DNase activities.

References:

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

NG dART RT-PCR Kit is a first strand cDNA synthesis kit convenient for two step RT-PCR. The kit consists of modified reverse transcriptase with improved thermostability (up to 65°C) and processivity. NG dART RT-PCR Kit has easy to use format to save time and limit the possibility of pipetting 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. **5 x NG cDNA Buffer** contains optimized for RT reaction 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 takes place in a separate tube - PCR reaction in which cDNA (as 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
5 x NG cDNA Buffer	100 µl	400 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
10 x Pol Buffer C with $MgCl_2$	250 µl	1.0 ml
RNase-free Water	1 ml	4 x 1 ml

EURx Ltd. 80-297 Gdańsk Poland ul. Przyrodników 3, NIP 957-07-05-191, KRS 0000202039
www.eurx.com.pl, orders@eurx.com.pl, *tel.* +48 58 524 06 97, *fax* +48 58 341 74 23

First strand cDNA synthesis:

1. Place 5 x 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:
5 x 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
10 x 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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