NG dART RT Kit

Cat. No.	size
E0801-01	25 reactions
E0801-03	50 reactions
E0801-02	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.

NG dART RT Kit is 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 Kit has easy to use format to save time and limit the possibility of pipetting errors.

NG dART RT 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. In PCR reaction cDNA (as a template) and specific primers are used to amplify double-stranded DNA of interest using the polymerase of choice. We strongly recommend using high fidelity OptiTaq DNA Polymerase (E2600-01) or OptiTaq PCR Master Mix (2x) (E2910-01).

COMPONENTS OF THE KIT

NG dART RT Kit	E0801-01	E0801-03	E0801-02
NG dART RT Mix	25 µl	50 µl	100 µl
5 x NG cDNA Buffer	100 µl	200 µl	400 µl
Oligo(dT)$_{20}$ (50 µM)	25 µl	50 µl	100 µl
Random hexamers (200 ng/µl)	25 µl	50 µl	100 µl
RNase-free Water	1 ml	2 x 1 ml	4 x 1 ml

References:

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 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.

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