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

NG dART RT 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 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 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
5X 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	1.0 ml	2 x 1.0 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.

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

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.