smART Reverse Transcriptase

Cat. No.	size
E1376-01	10 000 U
E1376-02	4 x 10 000 U

Unit Definition:

One unit incorporates 1 nmol of TTP into acid precipitable material in 10 min at 37°C using poly(A): oligo dT as a template: primer (1).

Storage Conditions: Store at -20°C.

Concentration: 200 U/µl

Quality Control:

All preparations are assayed for contaminating endonucleases, exonucleases, nonspecific RNases, 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.*

smART Reverse Transcriptase is a reverse transcriptase with reduced RNase H activity,increased thermal activity and processivity. smART is used for the preparation of cDNA libraries or for first strand cDNA synthesis for use in RT-PCR or RT-qPCR reactions.

Description:

- Reduced RNase H activity.
- Increased thermal stability in the range of 37°C to 65°C.
- Downstream application: PCR and qPCR.
- Ideal for cloning and diagnostic purposes targets up to 7 kb.

COMPONENT:	10 000 U
smART (200 U/µl)	50 µl
5 x cDNA Buffer	500 µl
0.1 M DTT	250 µl

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I First strand cDNA synthesis:

1. Place 5 x 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:
RNA (10 ng—5 µg)	x µl
primer*	1 µl
10 mM dNTP Mix Cat. No. E0503	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. *Optional*. If GC-rich or structured RNA template is used, mix gently, centrifuge briefly and incubate 5 min at 65°C then chill on ice.
4. Add the following components to the reaction tube in the indicated order:

Component:	Amount:
5 x cDNA Buffer	4 µl
DTT 0.1 M	2 µl
RNase Inhibitor 50 U/µl Cat. No. E4210	0.5 µl
smART (200 U/µl)	1 µl
Total volume	20 µl

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

Oligo(dT)$_{20}$ primed:	30-60 min at 50°C (or 37-65°C)
Gene specific primed:	30-60 min at 50°C (or 37-65°C)
Random hexamer primed:	25°C for 10 min, followed by 20-50 min at 50°C (or 37-65°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.

6. Terminate the reaction by incubating at 85°C for 5 min.
7. cDNA is ready for PCR, can be used immediately or stored at -20°C. Use 2-5 µl for 50 µl PCR.

II PCR

The product of the first strand cDNA synthesis can be used directly in PCR or qPCR. Normally, 2 µl of cDNA is used as template for subsequent PCR or qPCR in 25 µl total volume.

cDNA is compatible with various EURx PCR amplification products. For standard PCRs we recommend to use OptiTaq DNA Polymerase Cat. No. E2600 or depending on the downstream application tiOptiTaq DNA Polymerase Cat. No. E2725 or Perpetual OptiTaq DNA Polymerase Cat. No. E2720.

Regarding qPCRs cDNA is optimized for SYBR Green I dye (product Cat. No.: E0401, E0402, E0411, E0412) or for Probe detection (product Cat. No.: E0420, E0421, E0422, E0423).