smART RT-PCR Kit

Cat. No.	size
E0805-01	25 reactions
E0805-02	100 reactions

smART RT-PCR Kit is a complete system for efficient synthesis of first strand cDNA and PCR. Kit contains smART Reverse Transcriptase with reduced RNase H activity, increased thermostability and processivity. The second strand synthesis (PCR) is performed with recommended OptiTaq DNA Polymerase.

smART 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).

Description:

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

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.

COMPONENT:	E0805-01	E0805-02
smART (200 U/µl)	25 µl	100 µl
5 x cDNA Buffer	100 µl	400 µl
0.1 M DTT	50 µl	200 µl
Oligo $(dT)_{20}$ (50 µM)	25 µl	100 µl
Random hexamers (200 ng/µl)	25 µl	100 µl
10 mM dNTPs Mix	25 µl	100 µl
RNase Inhibitor (50 U/µl)	12.5 µl	50 µl
OptiTaq DNA Polymerase (2.5 U/µl)	25 µl	100 µl
10 x Pol Buffer C with $MgCl_2$	125 µl	500 µl
RNase-free Water	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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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	1 µl
RNase-free Water	to 12.5 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	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 or qPCR.

II 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 10 mM each	1 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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