

I. List of Components

Store all components at -20°C .

- 100 U/ μl SMARTScribe Reverse Transcriptase
- 5X First-Strand Buffer
- 20 mM DTT

II. Additional Materials Required

- RNase-free H_2O
- dNTP Mix (10 mM each); we recommend Advantage® UltraPure PCR Deoxynucleotide Mix (Cat. No. 639125)
- Oligo (dT)₁₂₋₁₈, random hexamer or gene-specific primers may be used.
- [Optional] 60 mM EDTA

III. For SMART® First-Strand cDNA Synthesis Reactions

Follow the protocol in the user manual for your specific SMART cDNA-based application. For the most up-to-date protocols, please visit our website at www.clontech.com.

IV. First-Strand cDNA Synthesis Reaction

This 10- μl reaction is suitable for synthesizing first-strand cDNA from 10 ng–1 μg of polyA⁺ RNA or 5 ng–2 μg of total RNA. If you are using more than 2 μg of RNA, prepare a 20- μl reaction containing double the volume of each component indicated below.

1. Add 1 μl of 20 μM primer stock (final concentration 2 μM) to your RNA sample. Add RNase-free H_2O to a final volume of 5 μl .
2. Heat the mixture at 72°C for 3 min, and immediately cool on ice.
3. Centrifuge briefly, then add the following:
 - 2 μl 5X First-Strand Buffer
 - 1 μl dNTP Mix
 - 1 μl 20 mM DTT
4. Mix the contents of the tube by gently pipetting up and down. Add 1 μl (100 U/ μl) SMARTScribe RT (100 U/ μl) and mix again.
5. Incubate at 42°C for 60–90 min.
6. Terminate the reaction by heating at 70°C for 15 min, or by adding 4 μl of 60 mM EDTA.

V. References

Ausubel, F. M., *et al.* (1995) In *Current Protocols in Molecular Biology* (Greene Publishing Associates, Inc. and John Wiley & Sons, Inc.), Supplement 29, Section 5.5.

Contact Us For Assistance	
Customer Service/Ordering	Technical Support
Telephone: 800.662.2566 (toll-free)	Telephone: 800.662.2566 (toll-free)
Fax: 800.424.1350 (toll-free)	Fax: 650.424.1064
Web: www.clontech.com	Web: www.clontech.com
E-mail: orders@clontech.com	E-mail: tech@clontech.com

Notice to Purchaser

Our products are to be used for research purposes only. They may not be used for any other purpose, including, but not limited to, use in drugs, *in vitro* diagnostic purposes, therapeutics, or in humans. Our products may not be transferred to third parties, resold, modified for resale, or used to manufacture commercial products or to provide a service to third parties without prior written approval of Clontech Laboratories, Inc.

Your use of this product is also subject to compliance with any applicable licensing requirements described on the product's web page at <http://www.clontech.com>. It is your responsibility to review, understand and adhere to any restrictions imposed by such statements.

Clontech®, the Clontech logo, Advantage, SMART, and SMARTScribe are trademarks of Clontech Laboratories, Inc. All other marks are the property of their respective owners. Certain trademarks may not be registered in all jurisdictions. Clontech is a Takara Bio Company. ©2015 Clontech Laboratories, Inc.

This document has been reviewed and approved by the Clontech Quality Assurance Department.