

STEMscript™ cDNA Synthesis Kit with Oligo(dT) Primers



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Catalog # 79003 1 Kit 100 Reactions

Product Description

STEMscript™ cDNA Synthesis Kit with Oligo(dT) Primers includes three components that have been optimized to provide efficient synthesis of first-strand cDNA in preparation for qPCR amplification: STEMscript™ Reverse Transcriptase Mix (contains STEMscript™ Reverse Transcriptase purified from recombinant E. coli, and recombinant ribonuclease inhibitor), STEMscript™ Reaction Buffer (contains oligo(dT) primers, MgCl₂, and dNTPs), and nuclease-free water. Oligo(dT) primers are ideal for reverse transcription of eukaryotic mRNA, as they anneal to poly(A) tails.

Ordering Information

PRODUCT NAME	CATALOG #	SIZE	COMPONENTS
STEMscript™ cDNA Synthesis Kit with Oligo(dT) Primers	79003	1 Kit - 100 Reactions	<ul style="list-style-type: none">• STEMscript™ Reverse Transcriptase Mix• Nuclease-Free Water• STEMscript™ Reaction Buffer with Oligo(dT) Primers

Component Storage and Stability

The following components are sold as part of a kit (Catalog #79003) and are not available for individual sale.

COMPONENT NAME	COMPONENT #	SIZE	STORAGE	SHELF LIFE
STEMscript™ Reverse Transcriptase Mix	79005	2 x 100 µL	Store at -20°C.	Stable until expiry date (EXP) on label.
Nuclease-Free Water	79006	2 x 1.25 mL	Store at -20°C. Alternatively, store at < 30°C.	Stable until expiry date (EXP) on label.
STEMscript™ Reaction Buffer with Oligo(dT) Primers	79007	2 x 200 µL	Store at -20°C.	Stable until expiry date (EXP) on label.

Materials Required But Not Included

PRODUCT NAME	CATALOG #
Total RNA purification kit	e.g. Qiagen 74104
PCR tubes or plate	e.g. Corning PCR-02-C or PCR-96-C
Filtered pipette tips	e.g. 38035
Microcentrifuge tubes, 0.65 mL	e.g. 38037
Thermocycler	---

Directions for Use

The following instructions are for preparing a final reaction volume of 20 μ L for reverse transcription. If preparing other volumes, adjust accordingly.

1. Purify RNA samples using a total RNA purification kit. Store on ice.
2. Thaw STEMscript™ Reverse Transcriptase Mix, Nuclease-Free Water, and STEMscript™ Reaction Buffer with Oligo(dT) Primers on ice. Mix gently, then centrifuge briefly.
3. Prepare 10 μ L Reagent Mix for each cDNA reaction by combining components on ice in the order listed in Table 1. Mix gently by pipetting after each addition.

Table 1. Reagent Mix Components

COMPONENT	VOLUME PER REACTION
Nuclease-Free Water	4 μ L
STEMscript™ Reaction Buffer with Oligo(dT) Primers	4 μ L
STEMscript™ Reverse Transcriptase Mix	2 μ L
Total volume	10 μL

4. Gently mix the Reagent Mix by pipetting or vortexing.
5. OPTIONAL: To denature RNA with a high degree of secondary structure (> 60% GC content), incubate RNA samples at 70°C for 5 minutes, then immediately chill in ice water for at least 5 minutes. Centrifuge for 5 seconds at maximum speed.
6. In each reaction tube or well of a reaction plate on ice, combine 10 μ L Reagent Mix with up to 10 μ L of RNA sample. Bring to a final volume of 20 μ L with Nuclease-Free Water.
7. Mix each reaction by pipetting. Close or seal the reaction tubes or wells.
8. Incubate the reaction tubes in a programmed thermocycler, using the conditions indicated in Table 2.

Table 2. Thermocycler Conditions for Reverse Transcription

STEP	TEMPERATURE	TIME	NUMBER OF CYCLES
Anneal primer*	25°C	5 minutes	1
Extension	42°C**	60 minutes	1
Inactivation	70°C†	15 minutes	1
Hold	4°C	Infinite	1

*Optional

**May be optimized between 37 - 55°C.

†Alternatively, use 95°C for 5 minutes.

9. Store the reaction products at 2 - 8°C for up to 24 hours or at -20°C for long-term storage.

Related Products

For related products, including genome editing tools, qPCR arrays, specialized cell culture and storage media, and cultureware, visit www.stemcell.com or contact us at techsupport@stemcell.com.

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