HiFiScript 1st Strand cDNA Synthesis Kit

Cat. #: W2569-10 (10 reactions); W2569-30 (30 reactions); W2569-100 (100 reactions)

Storage: Ship at 4 ℃. Store at -20℃ for up to 1 year and avoid freeze-thaw cycles.

Product Components

Components	W2569-10	W2569-30	W2569-100
HiFiScript, reverse transcriptase, 200 U/μL	10 μL	10 μL x 3	100 μL
5X RT Buffer	50 μL	50 μL x 3	500 μL
Primer Mix	24 μL	24 μL x 3	240 μL
dNTP Mix, 2.5 mM Each	50 μL	50 μL x 3	500 μL
DTT, 0.1 M	24 μL	24 μL x 3	240 μL
RNase-Free H_2O	1 mL	1 mL	1 mL

Application

- Reverse transcription, 1st strand cDNA synthesis
- cDNA library construction

Product Description (This product is for research use only.)

HiFiScript 1st Strand cDNA Synthesis Kit has been designed for the first-strand cDNA synthesis kit in a two-step RT-PCR experiments. HiFiScript reverse transcriptase in the kit is a new recombinant Moloney Murine Leukemia Virus Reverse Transcriptase (M-MLV RT) with novel mutations, which endorse HiFiScript the following features:

- High transcriptional efficiency – higher yields of cDNA
- High sensitivity – pg level total RNA and/or mRNA can be used to synthesis first strand cDNA.
- RNase H activity is removed – improved the elongation ability of the enzyme, and increased affinity of transcriptase to RNA template. Generate first strand cDNA up to 12 kb.

The optimized RT Buffer make the reverse transcriptase a wider range of applications, and with high compatibility for subsequent PCR and quantitative PCR experiments.

Note:

1. The operation should avoid RNase contamination to prevent RNA degradation. It is recommended to use special RNA operation working area, equipment and supplies.
2. All pipet tips should be RNase/DNase–free.
3. Invert the tubes in the kit to mix gently, and spin briefly before open. Avoid bubbles.
4. Return the enzymes immediately to -20 ℃ after use.
5. If the amount of the starting RNA is less than 50 ng, we suggest to add RNase inhibitor (RNasin) in the reaction. RNasin can be ordered from us, Cat.# W0596.

Protocol

Note: 20 μL reaction system is for 1 ng-5 μg of total RNA. If total RNA > 5 μg, scale up the reaction system.

Procedure:

1. Thaw Template RNA、Primer Mix、dNTP Mix、DTT、RT Buffer、and RNase-Free H_2O and place them on ice.

2. Add the reaction system to 20 μL according to the following table.

Reagent	20 μL reaction	Final Concentration
dNTP Mix，2.5 mM Each	4 μL	500 μM Each
Primer Mix	2 μL	
RNA Template	x μL	50 pg ~ 5 μg
5x RT Buffer	4 μL	1X
DTT, 0.1 M	2 μL	10 mM
HiFiScript, 200 U / μL	1 μL	
RNase-Free H_2O	to 20 μL	-

Note: 1) If the starting RNA is less than 50 ng, RNase Inhibitor (RNasin, Cat.#: W0596) should be added.

2) Primer Mix contains Oligo (dT) and Random Primer.

1. Vortex mix and brief spin to collect the contents at the bottom of the tube.
2. Incubate at 42℃ for 30 ~ 50 minutes, and then 85℃ for 5 minutes. Place it on ice after a brief centrifugation.
3. Store at -20℃ or use it for PCR or real-time PCR reaction directly.

If the reverse transcription efficiency is low or RNA template is complex or has a rich GC content, please use the following protocol.

1. Thaw the RNA Template、Primer Mix、dNTP Mix、DTT、RT Buffer, HiFiScript and RNase-Free H_2O and place them on ice.
2. Add the reagents according to the following table, to the total volume of 13 μL.

Reagent	13 μl reaction	Final Concentration
dNTP Mix, 2.5 mM Each	4 μL	500 μM Each
Primer Mix	2 μL	
RNA Template	x μL	50 pg-5μg
RNase-Free H_2O	up to 13 μl	

3. Incubate at 70℃ for 10 minutes and immediately put on ice to incubate for 2 minutes.
4. Centrifuge briefly to collect the solution at the bottom of the tube.
5. Add the following reagents to the above reaction system.

Reagent	19 μl reaction	Final Concentration
5x RT Buffer	4 μl	1x
DTT，0.1 M	2 μl	10 mM

Note: 1）If the RNA content is less than 50ng, RNase inhibitor (RNasin, Cat.#: W0596) should be added.

2) Primer Mix contains Oligo (dT) and Random Primer.

6. Mix well gently, and incubate at 42℃ for 2 minutes.
7. Add 1μL HiFiScript（200 U / μL, mix well by gently pipetting up and down. Incubate at 42℃ for 50 minutes.
8. Incubate at 85℃ for 5 minutes. Place it on ice after a brief centrifugation.
9. Store at -20℃ or directly use for PCR or real-time PCR reaction directly.

-- The end –

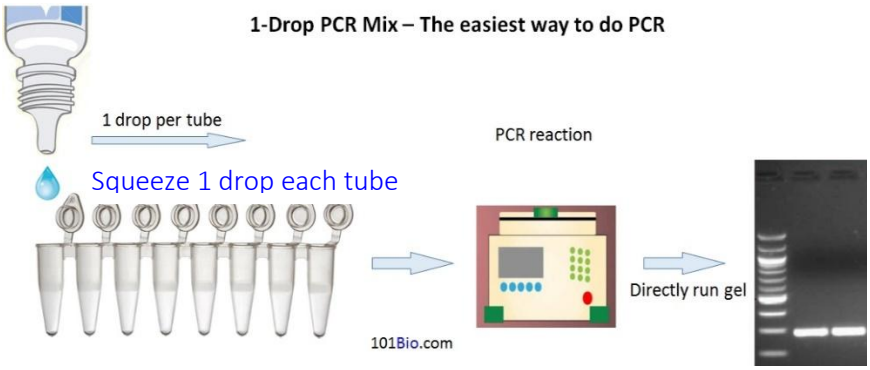
We offer good kits for rapid mouse genotyping as below.

① *Mouse Tail DNA extraction kit,* Crude Lysis. *Cat. # T605 / Fisher Sci. # NC1596514*

② *Mouse Tail Genomic DNA Kit,* Purified Lysis *Cat. # W2094*

③ Squeeze-1-Drop Do PCR *Master mix* *Cat. # 2599-5 / Fisher # NC1100436*

PCR Mix in dripping bottle



101Bio best-selling products.

Lentivirus / Retrovirus	Virus Concentration Solution Cat. # P904C FisherSci # NC3242189
10x Virus Titer-Up Booster	Increases virus titer up to 10-folds # P906 / P909 / Fisher # NC1792390
Lentiviral Expression Vectors	4 promoters: SFFV, CAG, CMV, EF1. 4 selections
Water Bath / Tub Disinfection	1 tablet cleans for 1 week / treats 1-4 gallons. Cat. # T20
Better than Matrigel	Real 3D Cell Culture Gel, 3-stiffness for different cells # P720

Cell Immortalization	SV40T / hTERT Cell Immortalization Kits. Simple & Reliable
Plasmid Miniprep	Endotoxin-Free Plasmid Miniprep Kit Cat. # W2106-50
Mycoplasma Detection qPCR	Mycoplasma causes contamination in cell culture. cat. # T42030