



Xscript 1 + DNA-Y cDNA Synthesis Kit

Product Number: XSCRT

Product Description

Xscript 1 + DNA-Y cDNA Synthesis Kit (+ gDNA wiper) includes a new generation of reverse transcriptase Xscript 1 Reverse Transcriptase and Buffer optimized for reverse transcription, further improving the efficiency of 1st strand synthesis. The 5 × gDNA wiper Mix in the kit can quickly remove genomic DNA contamination at 42°C for 2 min, which makes the results more reliable and simplifies qPCR primer design process without the need to design primers across introns. The kit contains single-component reverse transcription primers Oligo (dT)₂₀VN and Random hexamers, which allows users to choose primers flexibly for subsequent experiments as required. The kit can be used to synthesize full-length cDNA (up to 20 kb) for cloning and other downstream experiments, as well as highly uniform cDNA for qPCR.

Components

Components	RNAINH-MC2	RNAINH-MC10
RNase-free ddH ₂ O	1 ml	1 ml
5 × gDNA wiper Mix	100 µl	200 µl
10 × RT Mix ^a	100 µl	200 µl
Xscript 1 Enzyme Mix ^b	100 µl	200 µl
Oligo (dT) ₂₀ VN	50 µl	100 µl
Random hexamers	50 µl	100 µl

a. It contains dNTPs

b. It contains RNase inhibitor

Storage

Store at -30 ~ -15°C and transport at ≤ 0°C

10 × RT Mix contains high concentration of DTT, which may precipitate at low temperatures. Please restore to room temperature, shake gently and mix thoroughly, wait to dissolve the precipitation again before use.

Application

It is applicable for reverse transcription of animal, plant and microbial RNA.

Notes

The life science business of Merck operates as MilliporeSigma in the U.S. and Canada.

Prevent RNase contamination

Please keep the experiment area clean; Wear clean gloves and masks, and use new centrifuge tubes, tips and other supplies to ensure experiment is RNase-free.

Primers selection

For PCR

- For eukaryotic RNA templates, generally, use Oligo (dT)₂₀VN to obtain the highest yield of full-length cDNA.
- Use gene specific primer (GSP) to obtain the highest specificity. However, switch to Oligo (dT)₂₀VN or Random hexamers if GSP fails in the 1st strand cDNA synthesis.
- Random hexamers have the lowest specificity. All RNA, including mRNA, rRNA and tRNA can be templates of Random hexamers.
- Random hexamers may be used when the target region has complex secondary structures or high GC content, or when the template is derived from prokaryote and Oligo (dT)₂₀VN or Gene Specific Primers (GSP) can not effectively guide cDNA synthesis.

For qPCR

- Mixing Oligo (dT)₂₀VN with Random hexamers according to the guide ratio can make the synthesis efficiency of cDNA in each region of mRNA equal, which helps to improve the authenticity and reproducibility of quantitative results.

Experiment Process

♦ If the product will be used for PCR

RNA Denaturation*

Mix the following components in a RNase-free centrifuge tube:

RNase-free ddH ₂ O	to 8 µl
Total RNA	10 pg - 5 µg
or Poly A ⁺ RNA	10 pg - 500ng

Incubate at 65°C for 5 min, place on ice rapidly and let rest for 2 min.

* The denaturation step helps to open the secondary structures to improve the first strand cDNA yield. For cDNA fragment longer than 3 kb, do not ignore the denaturation step.

Removal of genomic DNA

Mix the following components in a RNase-free centrifuge tube:

Mixture of previous step	8 µl
5 × gDNA wiper Mix	2 µl

Mix gently with a pipette. Incubate at 42°C for 2 min.

Removal of genomic DNA

Mix the following components in a RNase-free centrifuge tube:

Mixture of previous step	8 µl
5 × gDNA wiper Mix	2 µl

Mix gently with a pipette. Incubate at 42°C for 2 min.

Preparation of 1st strand cDNA synthesis reaction mixture

Mixture of previous step	10 µl
10 × RT Mix	2 µl
Xscript 1 Enzyme Mix	2 µl
Oligo (dT) ₂₀ VN	1 µl
or Random hexamers	
RNase-free ddH ₂ O	5 µl

Mix gently with a pipette.

▲ This product is also suitable for reverse transcription using gene-specific primers (GSP). To avoid the potential effect of gDNA wiper on GSP, please add gene-specific primers (2 pmol) to the mixture.

Perform the 1st strand cDNA synthesis reaction under the following conditions

25°C ^a	5 min
37°C ^b	45 min
85°C	5 sec

a. Only necessary when using Random hexamers. Please skip this step when using Oligo (dT)₂₀VN or Gene Specific Primer.

b. For template with complicated secondary structures or high GC content, the temperature can be increased to 50°C, which will benefit the yield.

The product can directly be used for PCR reactions, or store at -20°C and used within six months. It is recommended to store in aliquots at -70°C for a long-term storage, and cDNA should be avoided repeated freezing and thawing.

To place an order or receive technical assistance

In Europe, please call Customer Service:

France: 0825 045 645

Germany: 069 86798021

Italy: 848 845 645

Spain: 901 516 645 Option 1

Switzerland: 0848 645 645

United Kingdom: 0870 900 4645

For other countries across Europe, please call: +44 (0) 115 943 0840

Merck KGaA
Frankfurter Strasse 250
64293 Darmstadt, Germany

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[SigmaAldrich.com/offices](https://www.sigmaaldrich.com/offices)

For Technical Service, please visit:
[SigmaAldrich.com/techservice](https://www.sigmaaldrich.com/techservice)

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◇ If the product will be used for qPCR

Removal of genomic DNA

Mix the following components in a RNase-free centrifuge tube:

RNase-free ddH ₂ O	to 10 µl
5 × gDNA wiper Mix	2 µl
Total RNA	10 pg - 1 µg
or Poly A ⁺ RNA	10 pg - 100ng

Mix gently with a pipette. Incubate at 42°C for 2 min.

Preparation of 1st strand cDNA synthesis reaction mixture

Mix the following components in a RNase-free centrifuge tube:

Mixture of previous step	10 µl
10 × RT Mix	2 µl
Xscript 1 Enzyme Mix	2 µl
Oligo (dT) ₂₀ VN	1 µl
Random hexamers	1 µl
RNase-free ddH ₂ O	4 µl

Mix gently with a pipette.

▲ This product is also suitable for reverse transcription using gene-specific primers (GSP). To avoid the potential effect of gDNA wiper on GSP, please add gene-specific primers (2 pmol) to the mixture.

Perform the 1st strand cDNA synthesis reaction under the following conditions

37°C*	45 min
85°C	5 sec

* For template with complicated secondary structures or high GC content, the temperature can be increased to 50°C, which will benefit the yield.

The product can directly be used for PCR reactions, or store at -20°C for six months. It is recommended to store in aliquots at -70°C for a long-term storage, and cDNA should be avoided repeated freezing and thawing.

