

igScript™ Reverse Transcriptase



Catalog #	3344
Package Size	50,000 units
Volume	250 µl
Concentration	200 units/µl

Description

igScript™ Reverse Transcriptase (igScript™ RT) is a recombinant Moloney Murine Leukemia Virus (MMLV) reverse transcriptase with reduced RNase H activity. It is highly efficient at producing full-length cDNA from long RNA templates. igScript™ RT is an RNA-directed DNA polymerase which lacks 3' → 5' exonuclease activity and is capable of producing cDNA from as little as 50 pg of total RNA for real-time RT-PCR analysis and other applications.

Protein Purity

The physical purity of this enzyme is ≥98% as assessed by SDS-PAGE with Coomassie® blue staining (see figure below).

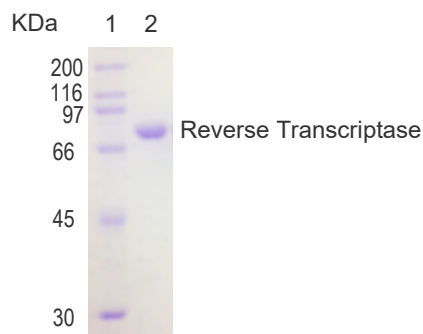


Fig: Lane 1. Protein marker
Lane 2. Reverse Transcriptase.

Product Source

The gene encoding recombinant MMLV Reverse Transcriptase with mutated RNase H domain is expressed in *E. coli*.

Applications

- First strand cDNA synthesis for PCR or RT-PCR
- Gene expression data validation by using RT-PCR product

Benefits

- Recombinant MMLV reverse transcriptase with greatly reduced RNase H activity.
- Active at temperatures up to 55°C.
- Highly efficient at producing full-length cDNA from as little as 50 pg of total RNA.

Product Includes

- 1) igScript™ Reverse Transcriptase
- 2) 10x igScript™ RT Reaction Buffer

Storage Temperature

-20°C

1x MMLV Reverse Transcriptase Reaction Buffer

50 mM Tris-HCl
75 mM KCl
3 mM MgCl₂
10 mM DTT
pH 8.3 @ 25°C

Storage Buffer

50 mM Tris-HCl, 50 mM KCl, 1 mM DTT, 0.1 mM EDTA, 50% Glycerol, pH 7.5 @ 25°C

Unit Definition

One unit is defined as the amount of enzyme required to incorporate 1 nmol of dTTP into acid insoluble material in 10 minutes at 37 °C using poly r(A)/oligo (dT) as a substrate.

Heat Inactivation

65°C for 20 minutes.

First Strand cDNA Synthesis Protocol

1. In a sterile micro-centrifuge tube, add the following components on ice:

Component	Volume
Total RNA	Up to 1.0 µg
Primer: d(T) ₂₃ VN (50 µM) or Random Primer Mix (60 µM) or Gene Specific Primer (10 µM)	2.0 µl
10 mM dNTPs	1.0 µl
H ₂ O	Up to 10.0 µl

2. Heat the reaction for 5 minutes at 65°C. Spin down briefly. Place the tube immediately on ice.
3. Add the following components:

Component	Volume
10x igScript™ RT Reaction Buffer	2.0 µl
igScript™ Reverse Transcriptase (200 U/µl)	1.0 µl
RNase Inhibitor (40 U/µl)*	0.2 µl
H ₂ O	6.8 µl

4. If using random hexamers, incubate the reaction at 25°C for 10 minutes, then proceed to step 5.
5. Incubate the reaction at 42°C for 30-60 minutes.
6. Inactivate the enzyme by incubating at 65°C for 20 minutes.
7. Store products at -20°C or proceed to next step.

* RNase Inhibitor, Murine (Cat.# 3714)

Related Products

- RNase Inhibitor, Murine (Cat.# 3714)
- igScript™ One Step RT-qPCR Kit (Cat.# 4214)
- igScript™ First Strand cDNA Synthesis Kit (Cat.# 4312)
- ig® SYBR Green qPCR 2x Master Mix (Cat.# 3354)
- igScript™ Probe Based One Step RT-qPCR Kit (Cat.#4243, 4245, 4247)



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Technical Support

Intact Genomics is committed to supporting the worldwide scientific research community by supplying the highest quality reagents. Each new lot of our products is tested to ensure they meet the quality standards and specifications designated for the product.

Please follow the instructions carefully and contact us if additional assistance is needed. We appreciate your business and your feedback regarding the performance of our products in your applications.