



igScript™ Reverse Transcriptase

Manual

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



Important!

-20°C Storage Required

- * Immediately inspect packages
- * Freeze upon receipt



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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).

Product Source:

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

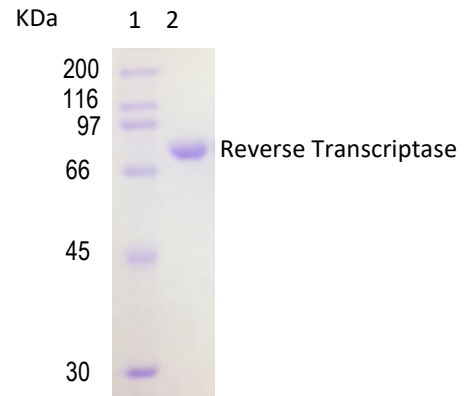


Fig: Lane 1. Protein marker

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 Components:

- igScript™ Reverse Transcriptase
- 10x igScript™ RT Reaction Buffer

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 Temperature:

-20°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)

Ordering Information:

- Order online within the USA. Place orders on **www.intactgenomics.com** using our secure Shopping Cart.
- Order by email, phone, or fax.
Email: **sales@intactgenomics.com**
Phone: (314) 942-3655 | Toll-free : 855-835-7172 | Fax: (314) 942-3656
- Order via our distributors.

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Our hours are Monday - Friday, 8AM to 5PM, U.S. Central Standard Time.

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