

igScript™ Reverse Transcriptase

Catalog #	3342	3344
Package Size	10,000 units	50,000 units
Volume	50 µl	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).

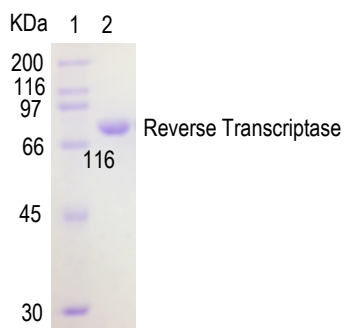


Fig: Lane 1. Protein marker
Lane 2. Reverse Transcriptase.

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 includes

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 can be purchased from any commercial source.