



# igScript™ First Strand cDNA Synthesis Kit

<b>Catalog #</b>	4312	4314
<b>Package Size</b>	25 reactions	100 reactions

## Description

igScript™ first strand cDNA synthesis kit includes 5x igScript™ master mix which contains igScript™ Reverse Transcriptase, recombinant RNase inhibitor, dNTPs, an optimized buffer, MgCl<sub>2</sub> and protein stabilizers. igScript™ Reverse Transcriptase is a recombinant MMLV reverse transcriptase with reduced RNase H activity and increased thermostability. The kit also provides two optimized primers and nuclease-free water. An anchored Oligo-dT primer [d(T)<sub>23</sub>VN] forces the primer to anneal to the beginning of the polyA tail and the random hexamer primer mix provides random and consistent priming sites covering the entire RNA templates including both mRNAs and non-polyadenylated RNAs. The kit is highly efficient at producing full-length cDNA from long RNA templates at temperatures between 42-55 °C.

## Applications

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

## Benefits

- 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) 5x igScript™ master mix
- 2) Oligo d(T)<sub>23</sub> VN primer (50 µM)
- 3) Random hexamer primer mix (60 µM)
- 4) Nuclease free water

## Storage Temperature

-20 °C

## 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
5x igScript™ Master Mix	4.0 µl
Primer: d(T) <sub>23</sub> VN (50 µM) and/or random primer mix (60 µM) or Gene specific primer (10 µM)	2.0 µl
Nuclease free H <sub>2</sub> O	Up to 20.0 µl

2. If using random hexamers, incubate the reaction mixture at 25°C for 10 minutes, then proceed to step 3.
3. Incubate the reaction mixture at temperatures between 42° C to 55°C for 30-60 minutes.
4. Inactivate the reaction by incubating at 65°C for 20 minutes.
5. Proceed to PCR amplification step.