igScript™ RT-PCR kit

Catalog #	4413	4415
Package Size	50 reactions	200 reactions

Description

igScript™ RT-PCR Kit combines two powerful mixtures: i). 5x igScript™ master mix and ii) Hot start *Taq* 2x master mix with standard buffer for 2-steps RT-PCR. The two mixtures require minimal handling during reaction setup and offer consistent and robust RT-PCR reactions.

The first strand cDNA synthesis is achieved by using 5x igScript™ master mix which contains igScript™ Reverse Transcriptase, recombinant RNase inhibitor, dNTPs, an optimized buffer, $MgCl_2$ 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 [$d(T)_{23}$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 -55ºC.

The amplification step features a high quality Hot start *Taq* DNA Polymerase which offers higher fidelity and better amplification in a master mix format. RT-PCR product can be generated up to 6 kb.

Applications

- cDNA synthesis followed by gene expression data validation .

Benefits

- Robust and active for cDNA synthesis 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 $d(T)_{23}$ VN primer (50 µM)
3) Random hexamer primer mix (60 µM)
4) Hot start Taq 2x master mix
5) Nuclease free water

Storage Temperature

–20 °C

Protocol

(A). First strand cDNA synthesis

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: $d(T)_{23}$VN (50 µM) and/or random primer mix (60 µM) or Gene specific primer (10 µM)	2.0 µl
Nuclease free H_2O	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 55ºC for 30-60 minutes.
4. Inactivate the reaction by incubating at 65°C for 20 minutes.
5. Proceed to PCR amplification step.

(B). PCR amplification

1. Prepare a reaction mix according to the following table:

PCR reaction set up:	
Diluted cDNA	1-5 µl
Forward primer (5 µM)	1.0 µl
Reverse primer (5 µM)	1.0 µl
Hot start *Taq* 2x master mix	10.0 µl
H_2O up to	20.0 µl

2. Mix the reaction mixture thoroughly.
3. Program the thermal cycler according to the manufacturer's instructions.
4. A typical PCR cycling program is outlined in the following table.
5. Place the PCR tubes in the thermal cycler and start the cycling program.

PCR cycling conditions:			
Steps	Temperature	Time	Cycles
Initial denaturation	95ºC	15 min	1
Denaturation	94ºC	30 sec	25-40
Annealing	50-66ºC	30 sec	
Extension	72ºC	1 min/kb	
Final extension	72ºC	5 min	1

6. Analyze 5 µl of PCR products by agarose gel electrophoresis.

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