igScript™ RT-qPCR Kit

Catalog #	4513	4515
Package Size	50 reactions	200 reactions

Description

igScript™ RT-qPCR kit combines two powerful mixtures: i). igScript™ first strand cDNA synthesis kit and ii) ig SYBR® Green qPCR 2x master mix with standard buffer providing improved PCR efficiency, wider dynamic range, superior sensitivity and specificity. The two mixtures require minimal handling during reaction setup and offer consistent and robust RT-qPCR reactions.

igScript™ first strand cDNA synthesis kit includes 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 42-55 ºC.

ig SYBR® Green qPCR 2x master mix is a ready-to-use cocktail containing all components except primers and template, for the amplification and detection of DNA in qPCR. The ig SYBR® Green qPCR 2x master mix with integrated chemically-modified hot start *Taq* polymerase, SYBR® Green I fluorescent dye, $MgCl_2$, dNTPs and stabilizers. ROX reference dye is not included in the 2x master mix*. The amplification step features a high quality hot start *Taq* DNA Polymerase which offers higher fidelity and better amplification.

Applications

- Gene expression data validation.
- Multiplexing
- Mutation detection
- Pathogen and viral detection
- Genetically modified organisms (GMO) characterization and Genetic profiling

Benefits

- Enhanced efficiency, specificity, and sensitivity
- Compatible with all real-time PCR instruments
- Superior gene expression results under various cycling conditions
- Robust and active for cDNA synthesis at temperatures up to 55°C.

Product Includes

1) 5x igScript™ master mix
2) Oligo $d(T)_{23}$ VN primer (50 µM)
3) Random hexamer primer mix (60 µM)
4) ig SYBR® Green qPCR 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. Place all kit components and cDNA samples on ice.
2. Mix and then centrifuge briefly to collect contents at the bottom of the tube.
3. Prepare a master mix for each reaction and control reaction plus 10% extra to allow for pipetting error 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
igSYBR Green qPCR 2x master mix	10.0 µl
ROX (option)	0.4 µl
H_2O up to	20.0 µl

4. Mix the reaction mixture thoroughly.
5. Program the thermal cycler according to the manufacturer's instructions.
6. A typical PCR cycling program is outlined in the following table.
7. 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	95ºC	5 sec	25-40
Annealing/Extension**	~60ºC	30 sec	
Melting curve analysis	According to instrument guidelines		

8. Analyze the data according to manufacturer protocol.

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*The use of ROX dye is necessary for all Applied Biosystems instruments and is optional for the Stratagene Mx3000P™, Mx3005P™, and Mx4000™ cyclers. Bio-Rad, Qiagen, Eppendorf, Illumina and Roche instruments do not require ROX dye.

** For 3 step cycling protocols, anneal at optimal annealing temperature for 30 sec followed by the minimum time required for data acquisition at 72 ºC according to instrument guidelines.

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