igScript™ Probe-Based RT-qPCR Kit

Manual

Catalog #	4243	4245	4247
Package Size	50 reactions	100 reactions	500 reactions
Volume	20µl		

Important!

-20°C Storage Required

* Immediately inspect packages
* Freeze upon receipt

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Intact Genomics, Inc.

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Description:

igScript™ Probe-Based RT-qPCR Kit combines two powerful mixtures: i). igScript™ First Strand cDNA Synthesis Kit and ii) igScript™ Probe-Based 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, 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 42-50 ºC.

igScript™ Probe-Based qPCR 2x Master Mix is a ready-to-use cocktail containing all components except primers and template, for the amplification and detection of DNA. This 2x master mix includes proprietary buffer, *Taq* DNA polymerase, low ROX reference dye, $MgCl_2$, dNTPs, stabilizers and enhancers.

Product Components:

- 5x igScript™ Master Mix
- Oligo $d(t)_{23}VN$ primer (50 µM)
- Random Hexamer primer mix (60 µM)
- igScript™ Probe-Based qPCR 2x Master Mix
- Nuclease-free water

Storage Temperature:

-20°C

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 50°C.

Protocol:

(A). First strand cDNA synthesis:

1. In 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 water	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 50°C for 30-60 minutes.
4. Inactivate the reaction by incubating at 65°C for 20 minutes.
5. Store products at -20°C or proceed to next 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	1.0-5.0 µl
Forward primer (5 µM)	1.0 µl
Reverse primer (5 µM)	1.0 µl
Probe (5 µM)	0.5 µl
igScript™ Probe-Based qPCR 2x Master Mix	10.0 µl
H_2O up to	20 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:

PCR Cycling Conditions			
Steps	**Temperature**	**Time**	**Cycles**
Initial denaturation	95ºC	3 min	1
Denaturation	95ºC	10 sec	40
Annealing/ Extension*	55-60ºC	30 sec	
Melting curve analysis	According to instrument guidelines		

7. Place the PCR tubes in the thermal cycler and start the cycling program.
8. Analyze the data according to the manufacturer protocol.

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

Related Products:

- igScript™ One Step RT-PCR Kit (Cat.# 4211)
- igScript™ One Step RT-qPCR Kit (Cat.# 4214)
- igScript™ Probe-Based qPCR 2x master mix (Cat.# 4233, 4235, 4237)
- igScript™ Probe-Based One Step RT-qPCR Kit (Cat.#4243, 4245, 4247)
- igScript™ First Strand cDNA Synthesis Kit (Cat.# 4312)
- igScript™ Reverse Transcriptase (Cat.# 3344)
- ig® SYBR Green qPCR 2x Master Mix (Cat.# 3354)

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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