

igScript™ Probe-Based RT-qPCR Kit



Catalog #	Package Size
4243	50 reactions
4245	100 reactions
4247	500 reactions

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, MgCl₂ 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)₂₃VN] 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 °C.

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, MgCl₂, dNTPs, stabilizers and enhancers.

Product Includes

- 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 ₂ O 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	95°C	3 min	1
Denaturation	95°C	10 sec	40
Annealing/ Extension*	55-60°C	30 sec	
Melting curve analysis	According to instrument guidelines		



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

** For 3 step cycling protocols, anneal at optimal annealing temperature for 30 sec followed by the minimum time required for data acquisition at 72°C 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)

Technical Support

Intact Genomics is committed to supporting the worldwide scientific research community by supplying the highest quality reagents. Each new lot of our products is tested to ensure they meet the quality standards and specifications designated for the product.

Please follow the instructions carefully and contact us if additional assistance is needed. We appreciate your business and your feedback regarding the performance of our products in your applications.

