



# igScript™ Probe-Based One Step RT-qPCR Kit

## Manual

Catalog #	4212	4215	4217	4219
Package Size	200 reactions	500 reactions	1,000 reactions	5,000 reactions
Volume	20µl			



### Important!

#### **-20°C Storage Required**

- \* Immediately inspect packages
- \* Freeze upon receipt



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## Description:

igScript™ Probe-Based One Step RT-qPCR 2x Master Mix contains igScript™ Reverse Transcriptase, *Taq* DNA polymerase, MgCl<sub>2</sub>, dNTPs, stabilizers and low ROX reference dye with standard buffer providing improved RTqPCR efficiency, wider dynamic range, superior sensitivity and specificity. igScript™ Probe-Based One Step RT-qPCR 2x master mix is a ready-to-use cocktail containing all components except primers, probe and template, for the amplification and detection of DNA in RT-qPCR. This 2x master mix requires minimal handling during reaction setup and offer consistent and robust RT-qPCR reactions. igScript™ Reverse Transcriptase is a recombinant MMLV reverse transcriptase with reduced RNase H activity, increased thermostability and can produce cDNA from small amount of total RNA for real-time RT-qPCR analysis and other applications. *Taq* DNA Polymerase is a thermostable DNA polymerase that possesses a 5'→3' polymerase (1, 2) and a 5'→3' exonuclease activity (3, 4). The amplification step features a high quality *Taq* DNA Polymerase which offers robust, reliable and better amplification.

## Product Components and Storage:

- igScript™ Probe-Based One Step RT-qPCR 2x Master Mix
- -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 55°C.

## Protocol:

1. Place kit components and RNA 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 plus 10% extra to allow for pipetting error, according to the following table:

PCR Reaction Set Up:	
RNA template	Up to 1.0 µg
Forward primer (5 µM)	1.0 µl
Reverse primer (5 µM)	1.0 µl
Probe (5 µM)	0.5 µl
One step RT-qPCR 2x master mix	10 µl
H <sub>2</sub> O 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:

PCR Cycling Conditions			
Steps	Temperature	Time	Cycles
First strand cDNA synthesis	42°C	30-60 min	1
Initial denaturation/ RT inactivation	95°C	3 min	1
Denaturation	95°C	5 sec	35-40
Annealing/ Extension*	~60°C	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 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 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™ 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.

## References:

1. Chien, A., Edgar, D. B. and Trela, J. M. (1976). J. Bact. 127, 1550-1557.
2. Lawyer, F. C. et al. (1993). PCR Methods and Appl. 2, 275- 287.
3. Longley, M. J., Bennett, S. E. and Mosbaugh D. W. (1990). Nucleic Acids Res.18, 7317-7322.
4. Lyamichev, V., Brow, M. A. and Dahlberg, J. E. (1993). Science. 260, 778-783.

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Our hours are Monday - Friday, 8AM to 5PM, U.S. Central Standard Time.

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