igScript™ Probe-Based One Step RT-qPCR Kit

Intact Genomics®

Catalog #	Package Size
4213	100 reactions
4215	500 reactions
4217	1,000 reactions
4219	5,000 reactions

Description

igScript™ Probe-Based One Step RT-qPCR 2x Master Mix contains igScript™ Reverse Transcriptase, Taq DNA polymerase, $MgCl_2$, dNTPs, stabilizers and low ROX reference dye with standard buffer providing improved RT-qPCR 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 Includes

- igScript™ Probe-Based One Step RT-qPCR 2x Master Mix

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.

Storage Temperature

-20 °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_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:

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

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.

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)

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.