Intact Genomics®

igScript™ One Step RT-qPCR Kit

Catalog #	4214	4218
Package Size	100 reactions	500 reactions

Description

igScript™ one step RT-qPCR kit combines two powerful mixtures: i). igScript™ Reverse Transcriptase 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™ Reverse Transcriptase is a recombinant MMLV reverse transcriptase with reduced RNase H activity and increased thermostability. The kit is highly efficient at producing full-length cDNA from long RNA templates at temperatures between 42-55ºC.

Intact Genomics 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 DNA polymerase, SYBR® Green I fluorescent dye, ROX dye*, MgCl2, dNTPs and stabilizers. This master mix is ideal for high-throughput real-time PCR screening and validation. 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) ig Script™ Reverse Transcriptase
2) ig SYBR™ Green qPCR 2x Master Mix

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 requiring Reverse Transcriptase enzyme 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
ig™ SYBR Green qPCR 2x Master Mix	10.0 µl
± Reverse Transcriptase	0.25 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 synthesis	42ºC	30-60 min	1
Initial denaturation	95ºC	15 min	1
Denaturation	94ºC	30 sec	25-40
Annealing	50-66ºC	30 sec	
Extension	72ºC	1 min/kb	
Final	72ºC	5 min	1

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.