igScript™ One Step RT-PCR Kit

Life Technologies™
Your Molecular & Cell Technology Partner

Intact Genomics®

Catalog #	4211	4213
Package Size	100 reactions	500 reactions

Description

igScript™ One Step RT-PCR Kit combines two powerful mixtures: i) IgScript™ Reverse Transcriptase and ii) Hot Start *Taq* 2x Master Mix 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-PCR 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.

Hot start *Taq* 2x Master Mix is a ready-to-use cocktail containing all components except primers and template, for the amplification and detection of DNA in PCR. The Hot Start *Taq* 2x Master Mix contains standard buffer, chemically-modified hot start *Taq* polymerase, $MgCl_2$, dNTPs and stabilizers. 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) igScript™ Reverse Transcriptase
2) Hot Start *Taq* 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
Gene specific forward primer (5 µM)	1.0 µl
Gene specific reverse primer (5 µM)	1.0 µl
Hot start *Taq* 2x master mix	10.0 µl
± Reverse Transcriptase	0.25 µl
H_2O up to	20.0 µl

4. Prepare a master mix for each control requiring NO Reverse Transcriptase enzyme plus 10% extra to allow for pipetting error according to the following table:
5. Mix the reaction mixture thoroughly.
6. Program the thermal cycler according to the manufacturer's instructions.
7. 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 extension	72ºC	5 min	1

8. Place the PCR tubes in the thermal cycler and start the cycling program.
9. Analyze 5-10 µl of PCR products by agarose gel electrophoresis.

Related Products

- igScript™ One Step RT-qPCR Kit (Cat.# 4214)
- igScript™ Reverse Transcriptase (Cat.# 3344)
- igScript™ Probe Based One Step RT-qPCR Kit (Cat.# 4212)
- Hot Start Taq 2x Master Mix (Cat.# 3296)

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.

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