Intact Genomics®

igScript™ One Step RT-PCR Kit

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) ig Script™ 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:
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:

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

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.

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