



igScript™ One Step RT-PCR Kit

Manual

Catalog #	4211	4213
Package Size	100 reactions	500 reactions
Volume	20 µl	



Important!

-20°C Storage Required

- * Immediately inspect packages
- * Freeze upon receipt



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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 42-55°C. 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, MgCl₂, 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 Components and Storage:

- igScript™ Reverse Transcriptase
- Hot Start *Taq* 2x Master Mix
- -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 <i>Taq</i> 2x master mix	10.0 µl
± Reverse Transcriptase	0.25 µl
H ₂ O 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	42°C	30-60 min	1
Initial denaturation	95°C	15 min	1
Denaturation	94°C	30 sec	25-40
Annealing	50-66°C	30 sec	
Extension	72°C	1 min/kb	
Final extension	72°C	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)

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.

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

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