



Product Information

Version 1.1.13

Product: iScript One-Step qRT-PCR Kit

Code	Product Name
A4223	iScript One-Step qRT-PCR Kit-ROX, 1x1.25ml, 100RXN (20ul)
A4224	iScript One-Step qRT-PCR Kit-Low ROX, 1x1.25ml, 100RXN (20ul)
A4225	iScript One-Step qRT-PCR Kit-iCycler, 1x1.25ml, 100RXN (20ul)
A4226	iScript One-Step qRT-PCR Kit, 1x1.25ml, 100RXN (20ul)

Product Description

Biomatik's iScript One-Step qRT-PCR Kit is a complete qPCR system containing all necessary reagents for both reverse transcription and PCR amplification to occur in a single qPCR reaction tube. Specifically, this One-Step qRT-PCR kit contains a qRT-PCR Enzyme Mix and an iGreen qPCR MasterMix. Our proprietary qRT-PCR Enzyme Mix contains stabilizers and enhancers to optimize the two reactions in a real-time "single step". This One-Step qRT-PCR kit offers the end-users an efficient, easy to use and reliable alternative to conventional "two-step" sequential qRT-PCR. Gene-specific primers must be used along with this kit.

Biomatik's iScript One-Step qRT-PCR Kit has many advantages over similar competitors' products. The kit utilizes a mutated recombinant M-MuLV Reverse Transcriptase, which exhibits limited RNase H activity. RNaseOFF Ribonuclease Inhibitor and a proprietary RTase additive are blended into the RTase's storage buffer to protect RNA template from degradation, and to improve the fidelity during reverse transcription. The components of iGreen qPCR MasterMix have been developed for superb performance in sensitivity, signal-to-noise ratio, and complete elimination of primer dimers. The chemically modified HotStart Taq polymerase, included in iGreen qPCR MasterMix, significantly reduces non-specific PCR amplification observed with regular Taq polymerase. The user-friendly one-step/single tube setup minimizes the chance of introducing human-error and contamination. Overall, Biomatik's iScript One-Step qRT-PCR Kit demonstrates market leading sensitivity, efficiency, and reliability.

Due to variations in qPCR instruments, we offer different iProbe qPCR Mastermix formulations optimized for different machines. Please use the following table as a guideline for the selection of qPCR Mastermix appropriate for your particular instrument model.

Code	Product Name	qPCR Instruments
A4223	iScript One-Step qRT-PCR Kit-ROX, 1x1.25ml, 100RXN (20ul)	-ABI [®] 7000,7300,7700,7900, StepOnePlus [™] StepOne [™] -Eppendorf [®] Realplex 4
A4224	iScript One-Step qRT-PCR Kit-Low ROX, 1x1.25ml, 100RXN (20ul)	-ABI [®] 7500-Stratagene [®] Mx3000, Mx3005, Mx4000
A4225	iScript One-Step qRT-PCR Kit-iCycler, 1x1.25ml, 100RXN (20ul)	-BioRad [®] iCycler [®] , iQ [™] 5, MyiQ [™]
A4226	iScript One-Step qRT-PCR Kit, 1x1.25ml, 100RXN (20ul)	-BioRad [®] CFX96 -Roche LightCycler [®] 480 -MJ Research Opticon [™] and Opticon [™] 2 -MJ Research Chromo [®] 4 -Corbett Rotor-gene [®] 600,3000 -Eppendorf [®] Realplex 2

Shipping and Storage

Store all components at -20°C in a non-frost-free freezer. All components are stable for 1 year from the date of shipping when stored and handled properly.

Protocol

RT-PCR should be assembled in a nuclease-free environment. RNA sample preparation, reaction mixture assembly, PCR and subsequent reaction analysis should be performed in separate areas. The use of “clean”, automatic pipettors designated for PCR and aerosol resistant barrier tips are recommended.

1. Prepare a reaction mixture using the following:

Components	Volume/Reaction			Final Concentration
	10µl	20µl	50µl	
Total RNA or Poly(A) +mRNA	Variable	Variable	Variable	2pg - 0.2µg/rxn 0.01pg - 2ng/rxn
iGreen Mastermix	5µl	10µl	25µl	1X
qRT-PCR Enzyme Mix (50X)	0.2µl	0.4µl	1µl	1X
Forward Primer (6µM)	0.5µl	1µl	2.5µl	300nM
Reverse Primer (6µM)	0.5µl	1µl	2.5µl	300nM
Nuclease-free H ₂ O	Up to 10µl	Up to 20µl	Up to 50µl	-

Note:

- Gene Specific primers must be used.
- Amplicon should be <150bp in size.

2. Gently mix and ensure all the components are at the bottom of the amplification tube. Centrifuge briefly if needed.

3. Program the thermal cycler so that cDNA synthesis is followed immediately by qPCR amplification.

Step	Temperature	Duration	Cycles
cDNA Synthesis	42°C	30mins	1
Pre-Denature	95°C	10mins	1
Denature	95°C	15secs	40
Anneal	60°C	60secs	
Melting curve	According to the instrument guidelines		

Recommendations for Optimal Results

- Aliquot reagents to avoid contamination and to avoid repeated freeze-thaw cycles
- iScript One-Step qRT-PCR Mastermix components are light sensitive; avoid exposure to light
- Start PCR as soon as the reaction mixture is prepared and always keep the reaction mixture chilled in an ice box prior to qRT-PCR reactions

*** The kit is designed for laboratory research purpose only. Not for human or animal diagnostic and therapeutic use.**