



Qscript Iyo 1-step

Cat. No. 95198-024

24 reactions

Store at 2°C - 8°C
Protected from light

Description

Qscript Iyo 1-step is a lyophilized single-reaction reagent optimized for highly sensitive and reproducible one-step RT-qPCR using hydrolysis probes. The reagent contains a hot-start thermo-stable polymerase, a genetically engineered reverse transcriptase as well as other components to ensure higher performance detection of up to 5 targets with maximum sensitivity and specificity. The enhanced stability of the freeze-dried master mix enables convenient shipping and storage at room temperature. The single tube reaction facilitates easy reaction set up while preventing potential cross-contamination.

Instrument Compatibility

Different real-time PCR systems employ different strategies for the normalization of fluorescent signals and correction of well-to-well optical variations. It is critical to match the appropriate qPCR reagent to your specific instrument. The Qscript Iyo 1-step RT-qPCR Kit does not contain an internal reference dye. Please consult the following table, or visit our website at www.quantabio.com to find an optimized kit for your instrument platform(s).

Reagent	Cat Nos	Compatible Real-Time PCR Systems
Qscript Iyo 1-step	95198-024	Bio-Rad CFX96™, CFX384™, iCycler iQ®, iQ™5, MyiQ™ Opticon™, MiniOpticon™, Chromo4™ Cepheid Smart Cycler®; Qiagen/Corbett Rotor-Gene® Eppendorf Mastercycler® ep realplex Roche Applied Science LightCycler® 480

Components

Reagent

Qscript Iyo 1-step mastermix (lyosphere)

Reagents not supplied

- Primer and probes
- RNA template
- Nuclease free water
- Rox passive reference dye.

If a passive reference dye, such as Rox, is required for your assay, it can be purchased separately and used according to the instrument company's recommendation.

Storage and Stability

Store the Qscript Iyo 1-step in a constant temperature freezer at 4°C upon receipt.

The kit can also be stored at room temperature for up to 9 months.

For lot specific expiry date, refer to package label, Certificate of Analysis or Product Specification Form.

Guidelines for One-Step RT-qPCR

- Unseal the pouch containing the lyophilized RT-qPCR strip tube. Each tube contains enough reagents to perform one RT-qPCR reaction in a final volume of 25 μ L.
- Calculate the amount of primer/probe, RNA template and water needed for each reaction to obtain a total volume of 25 μ L (Table below). Premix all or add separately to the tube containing the lyophilized reagent¹. After adding all the components, wait for 5-10 sec or until the lysphere has completely dissolved, then mix by gently pipetting up and down 3-5 times. Quick spin to bring solution to the bottom and transfer the entire 25 μ L to a 96 well plate. Alternatively, premix primer/probe with water in a total volume of 20 μ L and add to the tube containing the lysphere. Wait for 5-10 sec or until the sphere has completely dissolved, pipet up and down 3-5 times, spin and then transfer the entire 20 μ L reaction mix to a 96 well plate. Finally, add 5 μ L RNA template to the well. Seal and centrifuge the plate briefly before loading on to the instrument.

Component	Amt/well (μ L)
Nuclease Free Water	(25 -x-y)
primer/probe	x
RNA	y
Final volume	25

¹ The lyophilized reagent does not contain Rox as passive dye, so check off this option when performing the analysis. Rox dye can be added separately with instruction from the instrument company.

- **Recommended PCR Cycling Conditions for one-step Annealing/Extension**

Step	$^{\circ}$ C	Time	Cycles
Reverse transcription	50	15 min	1
RT inactivation and Initial Denaturation	94	3 min	1
Denaturation	94	15 sec	40
Annealing/Extension ²	60	60 sec	

² Optimal temperature and time for Annealing/Extension need to be determined by end user. For 3 step protocols, anneal at desired temperature for 30 sec, followed by extension at 72 $^{\circ}$ C for 30 to 60 sec.

Quality Control

Kit components are free of contaminating DNase and RNase. The Qscript lyo 1-step RT-qPCR Kit is functionally tested in RT-qPCR. Kinetic analysis must demonstrate linear resolution over six orders of dynamic range ($r_2 > 0.995$) and a PCR efficiency $> 90\%$



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