Product Information Sheet

Product Name: QuanTASE One-Step Universal RT-qPCR Kit* and QuanTASE Two-Step Reagent Kit *

Concentration: 4X
Storage and Handling: Store at -20^{0}C upon arrival
Ordering Information:

Item Number	Total Volume Received	Quantity Received	Total number of reactions which can be obtained when using the following reaction sizes	
			20µL Reactions	10µL Reactions
QS-KIT-200	IT-MM-200-PC: 1mL, RT-200-PC: 100uL, DTT-100-200uL-PC: 200uL	IT-MM-200-PC: 1x1mL, RT-200-PC: 1x100uL, DTT-100-200uL-PC: 1x200uL	200	400
QS-KIT-1000	IT-MM-200-PC: 5mL, RT-200-PC: 500uL, DTT-100-200uL-PC: 1000uL	IT-MM-200-PC: 5x1mL, RT-200-PC: 5x100uL, DTT-100-200uL-PC: 5x200uL	1000	2000
QS-KIT-2000	IT-MM-200-PC: 10mL, RT-200-PC: 1000uL, DTT-100-200uL-PC: 2000uL	IT-MM-200-PC: 10x1mL, RT-200-PC: 10x100uL, DTT-100-200uL-PC: 10x200uL	2000	4000
QS-SR-100***	5X RTScript Buffer-1x0.4mL, 10mM dNTP Mix-1x0.1mL, 100mM DTT Solution-1x0.1mL, 100µM Oligo-(dT)20 primer-1x0.05mL, 100µM Random Hexamers-1x0.05mL, 40 Units/uL Rnase Inhibitor-1x0.01mL, 10ng/uL Positive Control RNA-1x0.01mL, Rnase-free Water-1x1.2mL		100	200
EVA-300uL****	EvaGreen Dye, 20X in Water; EVA: 2x150uL		200	400
ROX-300uL****	ROX Reference Dye, 20X in Water; ROX: 2x150uL		Determined my volume required in assay by machine	Determined my volume required in assay by machine

***This item can be purchased separately to perform a Two-Step Protocol: First Strand cDNA Synthesis followed by Amplification.
****This item can be purchased separately if dyes are required for qPCR assays.

Product Description:
The QuanTASE Kit from Empirical Bioscience is an RT-qPCR Universal Kit which contains the reagents and MasterMixes necessary for accurate detection of DNA or RNA. The reagents have been optimized for qPCR or RT-qPCR applications for use with both intercalating dye and Probe-based detection methods.
This kit contains Empirical RTScript™ (M-MLV) Reverse Transcriptase Enzyme Mix, InhibiTaq qPCR Mastermix, EvaGreen Dye, and ROX reference dye.

Empirical's QuanTASE Two-Step Reagent Kit contains all the components necessary to perform first strand cDNA synthesis with high sensitivity and efficiency using a two step RT-qPCR protocol. These reagents combined with the optimized buffers and reagents in the QuanTASE kits synthesize complementary DNA from single stranded RNA or DNA templates. Empirical's RTScript™ is sensitive, specific, and is capable of synthesizing highly structured and long cDNA fragments. Produced cDNA is appropriate for qPCR analysis. This kit is supplied with 5X RTScript™ Buffer, dNTP Mix (10mM) , DTT Stock Solution (100mM), Oligo-(dT)20 primer (100µM), Random Hexamers (100µM), RNase Inhibiter (40 units/µL), Positive Control RNA (10ng/µL), and RNase-free Water.

*** This product is for "Research Use Only. Not for use in diagnostic procedures".**
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Protocol for One-Step: The following reaction set up and general cycling conditions are recommended but can vary depending on the template and primers being used.

Reaction set-up for 20uL reaction volume Table 1:

Component	Volume	Final Concentration
4X InhibiTaq HotStart qPCR MasterMix	5 µl	1X
RTScript™, 200 units/uL#	0.5 µl	5 units/µl
Target Specific Primers and Probe	1µl	1X
100mM DTT#	1µl	5mM
Sample	X µl	100 ng to 1 pg
EvaGreen Dye, 20X in Water (if required)	1 µl	1X
ROX reference dye, 25µM (See Table 2)	See Table 2	See Table 2
Nuclease Free Water to volume	X µl	N.A.
Total	20 µl	

#Reactions need to be placed in cycler immediately following completion to maintain the integrity of the DTT and the RTScript™.

Table 2: ROX concentration recommendation for different instrument

Type	Instrument		ROX final concentration
	Company	Instrument Name	
No ROX	Roche	LightCycler 480, LightCycler 2.0	None
	BioRad	iCycler, MyiQ, MiQ 2, iQ 5, CFX-96, CFX-384, Chromo4, MJ Opticon, Option2, MiniOpticon	
	Qiagen	Roto-Gene Q, Roto-Gene3000, Roto-Gene 6000	
	Illumina	Eco RealTime PCR System	
	Eppendorf	Mastercycler realplex	
	Cepheid	SmartCyler	
Low ROX	ABI	7500, 7500 Fast	30nM
	Stratagene	MX4000P, MX3000P, MX3005P	
High ROX	ABI	5700, 7000, 7300, 7700, 7900, 7900HT, 7900HT Fast, StepOne, StepOne, Viia7	300nM

Thermal cycling conditions:

Table 3: Recommended Cycling Conditions

Cycling Step	Stage	No. of Cycles	Temperature	Holding Time
UNG Incubation	1	1	25°C	2 minutes
RT Incubation	2	1	55°C	15 minutes
Enzyme Activation	3	1	95°C	2 minutes
Amplification**	4	40	95°C	10 seconds
			60°C	60 seconds

**Temperature and Holding Time will be based off primer set used.

Protocol for Two-Step: The following reaction set up and general cycling conditions are recommended but can vary depending on the template and primers being used.

Step 1A: cDNA synthesis without denaturation. General guidelines per 20µl reaction for setup without sample denaturation include, See Table 4

Table 4: cDNA synthesis without denaturation.

Component	Stock Conc.	Final Conc.	20uL Assay
RNase-free water	-	-	Up to 20µL
RNA template	-	Total RNA: 10 pg-5 µg or mRNA: 10pg-500ng	X µL
Primer	100µM	Gene specific primer: 10-20pg (50-100ng) Oligo-dT_{20} primer or random: 50pmol	0.1-0.2 µL 0.5µL
5X RTScript Buffer	5X	1X	4µL
dNTP Mix	10mM	500µM each	1 µL
DTT stock solution	100mM	5mM	1 µL
RNase Inhibitor	40 units / µL	20 units	0.5 µL
RTScript™ Reverse Transcriptase	200 units / µL	100 units	0.5 µL

Use reaction mix immediately to preserve the integrity of the enzyme.

Step 1B (optional): cDNA synthesis with denaturation.

- First, prepare the Template/Primer mix using the Table 5. Incubate the mix for 5 min at 65-70°C.

Table 5: Template/Primer Mix

Component	Stock Conc.	Final Conc.	20uL Assay
RNase-free water	-	-	Up to 10µL
RNA template	-	Total RNA: 10 pg-5 µg or mRNA: 10pg-500ng	X µL
Primer	100µM	Gene specific primer: 10-20pg (50-100ng) Oligo-dt_{15-25} primer or random: 50pmol	0.1-0.2 µL 0.5µL

- Second, prepare a Reaction mix using Table 6.

Table 6: Reaction Mix

Component	Stock Conc.	Final Conc.	20uL Assay
RNase-free water	-	-	Up to 10µL
5X RTScript Buffer	5X	1X	4µL
dNTP Mix	10mM	500µM each	1 µL
@DTT stock solution	100mM	5mM	1 µL
%RNase Inhibitor	40 units / µL	20 units	0.5 µL
# RTScript™ Reverse Transcriptase	200 units / µL	100 units	0.5 µL

@Adding up to 5mM DTT may increase the yield and is recommended for individual optimization %Addition of 20-40 Units of RNase inhibiter per assay is recommended when using low amounts of starting RNA. #100 Units of enzyme is recommended for standard assays, but increased transcription levels may be achieved with increasing the amount of enzyme up to 200 units. Use reaction mix immediately to preserve the integrity of the enzyme.

- Third, Add 10µl of Reaction Mix to 10uL of Template/primer mix and pipette gently up and down on ice.

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Step 2: Incubation
Gene Specific primers: Incubate Reaction Mix for 30-60min at 50°C.
Oligo-dT or Random primers: Incubate Reaction Mix for 10min at 42°C followed by 30-60min at 50°C.

Step 3 (optional): Heat inactivation
Heat the mixture for 70°C for 10 min to inactivate Reverse Transcriptase.

Step 4(optional): RNA removal
Add 2 Units DNase-free RNase and incubate at 37°C for 20min.

The cDNA can now be used as a template in qPCR.

Reaction set-up for 20uL reaction volume Table 7:

Component	Volume	Final Concentration
4X InhibiTaq HotStart qPCR MasterMix	5 µl	1X
Target Specific Primers and Probe	1µl	1X
100mM DTT#	1µl	5mM
cDNA Template from Two-Step Protocol	X µl	100 ng to 1 pg
EvaGreen Dye, 20X in Water (if required)	1 µl	1X
ROX reference dye, 25µM (See Table 2)	See Table 2	See Table 2
Nuclease Free Water to volume	X µl	N.A.
Total	20 µl	

#Reactions need to be placed in cycler immediately following completion to maintain the integrity of the DTT.

Thermal cycling conditions:

Table 8: Recommended Cycling Conditions

Cycling Step	Stage	No. of Cycles	Temperature	Holding Time
UNG Incubation	1	1	25°C	2 minutes
RT Incubation	2	1	55°C	15 minutes
Enzyme Activation	3	1	95°C	2 minutes
Amplification**	4	40	95°C	10 seconds
			60°C	60 seconds

**Temperature and Holding Time will be based off primer set used.

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