Product Information Sheet

Product Name: QuantiTASE PLUS One-Step Universal RT-qPCR Kit*

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Concentration:

InhibiTaq™ Plus HotStart qPCR MasterMix: 4X
RTScript™ Reverse Transcriptase: 200U/uL
DTT: 100mM

Storage and Handling:

Upon arrival store at -20°C until provided expiration date. Minimize Freeze thaw to avoid loss of performance.

Product Description:

QuantiTASE PLUS Kit:
A 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 RTScript™ Reverse Transcriptase Enzyme Mix, InhibiTaq™ PLUS Hotstart qPCR Mastermix, and DTT.

Protocol:

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™ PLUS HotStart qPCR MasterMix	5 µl	1X
RTScript™, 200U/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™. Mix Gently, Do Not Vortex

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 plus, Viia7	300nM

***This product is intended for Research Use Only. This product is manufactured under ISO13485:2016 Quality System Requirements and is available for use as a Raw Material for use in IVD applications. Please contact Empirical Bioscience for further details.**

For SDS and Certificate of Analysis please visit www.Fortislife.com

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:

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:

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. Mix gently, do not vortex.

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

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: Reaction Set Up

Component	Volume	Final Concentration
4X InhibiTaq™ Plus HotStart qPCR	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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