NIPPON Genetics EUROPE
INNOVATION FOR YOU

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FastGene® PROBE One Step Mix Separate ROX

Technical Data Sheet

Product Description

Combining the the reverse transcription with a qPCR detection mix results in the optimal convenience protocol therefore lowering the risk of contamination by avoiding additional pipetting.

The reverse transcriptase implemented in this kit is a modified MuLV, engineered to fasten the process of turning the RNA to DNA. This enables a 10 to 20 min reverse trancsription rather than a 1 hour step using the wild-type enzyme.

Extensive research allowed us to create a buffer mixture optimal for the detection of multiple fluorophores within a mixture. This research also led to the development of buffer chemistries optimized for earlier C_T-values in a qPCR assay. Combining the earlier C_T-values with the multiplex, the FastGene® Probe One Step qPCR with separate ROX Mix is perfect for qPCR assays using Probes.

Product Applications

FastGene® Probe qPCR Mixes ideally suited for:

- Multiplex RT-qPCR
- Gene expression analysis (absolute and relative)
- Detection of low copy genes
- Quantification of viral loads or NGS libraries

Limitation of Use

This product is for in vitro research only and not for clinical diagnostics.

Product Specifications

Shipping and Storage

Prolonged exposure to light must be avoided. The mix is stable for 12 months at -20 °C and for at least 30 freeze thaw cycles. Freeze/thaw cycles can be avoided by storing the mix at 4 °C. The kit will remain fully active for 1 month at 4 °C.

Primer design

Please verify the specificity of the primer pair by blasting the template's organism (Primer-BLAST: http://www.ncbi.nlm.nih.gov/tools/primer-blast/). The primers should amplify an amplicon with 80 – 200 bp. Do not exceed 400 bp. Extension and annealing time can reduced by amplification of smaller amplicons. Using the default settings of primer3 (http://frodo.wi.mit.edu/primer3/), the melting temperature should be 60 °C.

Kit Codes and Components

LS47SRS	FastGene® PROBE One Step Separate ROX	10 rxns
LS4701SR	FastGene® PROBE One Step Separate ROX	100 rxns
LS4705SR	FastGene® PROBE One Step Separate ROX	500 rxns

Quick Notes

- FastGene® Probe 1-Step RT-qPCR Kit can replace any commercial Probe based qPCR mixture. The annealing temperature may need to be optimized to account for differences in buffer formulation.
- FastGene® Probe 1-Step RT-qPCR Kit has been optimized for multiplex qPCR
- FastGene® Probe 1-Step RT-qPCR Kit is a flexible Kit for all devices with separate ROX

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Contact & Support

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For information on product use limitations and licenses:
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Step 1: Prepare the PCR master mix

- Ensure that all reagents are properly thawed and mixed.
- Prepare a PCR master mix containing the appropriate volume of all reaction components common to all or a subset of the reactions to be performed.
- Calculate the required volumes of each component based

Component	20 µl rxn	Final conc.
PCR-grade water	Up to 20 µl	N/A
2X FastGene® PROBE One Step Mix	10 µl	1X
FastGene® PROBE Scriptase	0.2 µl	1X
Probe (10µM)	0.4 µl	200 nM
Forward Primer (10 µM)	0.8 µl	400 nM
Reverse Primer (10 µM)	0.8 µl	400 nM
Template RNA	1 pg - 1 µg total RNA >0.01 pg mRNA	As required

Step 2: Set up individual reactions

- Transfer the appropriate volume of PCR master mix, template and primer to individual PCR tubes/wells or a PCR plate
- Cap or seal individual reactions, mix and centrifuge briefly.

Step 3: Run the PCR

- Perform PCR with the following cycling protocol:

Step	Temperature	Duration	Cycles
Reverse Transcription	45 - 55 °C	10-20 min	1
Initial denaturation	95 °C	2 min[1]	1
Denaturation	95 °C	5 sec	40
Annealing & Elongation	60 - 65 °C	20-30 sec	
Melt analysis	optional: only when using hybridization probes.		

[1] Initial denaturation for 2 min at 95 °C is recommended for most assays. For GC-rich targets (>65% GC), 5 min at 95 °C may be used.

[2] An annealing temperature 5 °C lower than the calculated melting temperature (T_m) of the primer set is recommended as a first approach. If low yields and/or nonspecific amplification is obtained, an annealing temperature gradient PCR is recommended to determine the optimal annealing temperature of the primer pair.

Addition of reference dye ROX

The reference dye ROX comes in a concentration of 50 µM. Depending of the instrument listed below you might need to prepare a low ROX or High ROX Master mix.

ROX Concentration	1 mL PROBE One Step Mix
High (500 nM)	+ 20 µl of ROX Stock solution
Low (50 nM)	+ 2 µl of ROX Stock solution

Instrument compatibility

The list below shows the ROX concentration requirement of some instruments:

High ROX concentration (500 nM)

Manufacturer	Model
ThermoFisher Scientific	7000, 7300, 7700, 7900, 7900HT, 7900HT FAST, StepOne™, StepOne™plus

Low ROX concentration (50 nM)

Manufacturer	Model
Agilent	MX3000P, MX3005P, MX4000P
Analytik Jena	qTower
Bio-Rad	CFX96, CFX 384, Chromo4, MiniOpticon, Opticon, Opticon™ 2
Cepheid	SmartCycler
Eppendorf	Mastercycler ep realplex, Mastercycler ep realplex 2S
Fluidigm	BioMark
Hain Lifesciences	FluoroCycler®96
PCR^{max}	Eco™ 48
Qiagen	RotorGene™ 3000, RotorGene™ 6000, RotorGene™ Q
Roche	LightCycler® 480, LightCycler® 96, LightCycler® Nano
Takara	Thermal Cycler Dice®(TP800)
Techne	PrimeQ, Quantica
ThermoFisher Scientific	7500, 7500 FAST, Piko Real®, QuantStudio™12k Flex, ViiA7™