

# FastGene® PROBE One Step Mix

## 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 min reverse transcription 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 2 x qPCR Universal 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 in order to not bleach the DNA dye. 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 be 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

LS475LRS	FastGene® PROBE One Step Mix Lo-ROX	10 rxns
LS4701LR	FastGene® PROBE One Step Mix Lo-ROX	100 rxns
LS4705LR	FastGene® PROBE One Step Mix Lo-ROX	500 rxns
LS475HRS	FastGene® PROBE One Step Mix Hi-ROX	10 rxns
LS4701HR	FastGene® PROBE One Step Mix Hi-ROX	100 rxns
LS4705HR	FastGene® PROBE One Step Mix Hi-ROX	500 rxns
LS475NRS	FastGene® PROBE One Step Mix No-ROX	10 rxns
LS47501NR	FastGene® PROBE One Step Mix No-ROX	100 rxns
LS47505NR	FastGene® PROBE One Step Mix No-ROX	500 rxns

### Related Products

LS43SLRS	FastGene® IC Green One Step Mix Lo-ROX	10 rxns
LS4301LR	FastGene® IC Green One Step Mix Lo-ROX	100 rxns
LS4305LR	FastGene® IC Green One Step Mix Lo-ROX	500 rxns
LS43SHRS	FastGene® IC Green One Step Mix Hi-ROX	10 rxns
LS4301HR	FastGene® IC Green One Step Mix Hi-ROX	100 rxns
LS4305HR	FastGene® IC Green One Step Mix Hi-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 comes as Lo-ROX or Hi-ROX mix

### Contact & Support



For information on product use limitations and licenses:  
<http://nippongenetics.eu/contact/terms/>

For technical support please contact:  
[support@nippongenetics.eu](mailto:support@nippongenetics.eu)

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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 on the following table:

Component	20 µl rxn	Final conc.
PCR-grade water	Up to 20 µl	N/A
2X FastGene® PROBE One Step Mix	10 µl	1X
20X FastGene® Scriptase	1 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 °C	10 min	1
Initial denaturation	95 °C	2 min <sup>1</sup>	1
Denaturation	95 °C	5 sec	40
Annealing & Elongation	60 - 65 °C	20 - 30 sec	
Melt analysis	optional: only when using for hybridization probes.		

<sup>1</sup> 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.

<sup>2</sup> 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.

### 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
Qiagen	RotorGene™ 3000, RotorGene™ 6000, RotorGene™ Q
Takara	Thermal Cycler Dice®(TP800)
Techne	PrimeQ, Quantica
ThermoFisher Scientific	7500, 7500 FAST, Piko Real®, QuantStudio™12k Flex, ViiA7™

#### No ROX concentration

Manufacturer	Model
PCR <sup>max</sup>	Eco™ 48
Roche	LightCycler® 480, LightCycler® 96, LightCycler® Nano