FastGene® Probe One Step Mix with UDG

Product description

FastGene® Probe One Step Mix with UDG is a Probe-based One-step RT qPCR kit. It is provided in a ready-to-use 4X Master Mix format, requiring only the addition of Template RNA, Probes and Primers to initiate the reaction. The kit includes Chemically modified HOT-start Taq Polymerase and Thermolabile Uracil-DNA Glycosylase for enhanced specificity. Please note that a minimum initial denaturation time of 10 minutes is required to activate the Chemically modified HOT-start Taq Polymerase.

Product applications

FastGene® Probe One Step Mix with UDG ideally suited for:

- Fast quantification of RNA
- Analysis of gene deletion
- Pathogen identification
- High-throughput SNP genotyping
- Linkage analysis

Limitation of use

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

Product specifications

Shipping and storage

The mix can be stored for up to 12 months at -20 °C. To avoid freeze/thaw cycles the kit can be aliquoted. Prolonged exposure to light must be avoided.

Primer design

Please blast your primer sequences to verify the specificity of the primer pair. (Primer-BLAST: http://www.ncbi.nlm.nih.gov/tools/primer-blast/). The primers should amplify an amplicon with 80 – 200 bp. To ensure optimal amplification product length should not exceed 400 bp. For amplification of smaller amplicons, the extension and annealing time can reduced. Using the default settings of primer3 (http://frodo.wi.mit.edu/primer3/), the melting temperature should be 60 °C.

Kit Codes and Components		
LS48s	FastGene® OneStep Probe Kit with UDG	10 rxns
LS4801	FastGene® OneStep Probe Kit with UDG	100 rxns
LS4805	FastGene® OneStep Probe Kit with UDG	500 rxns

Instrument compatibility

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

High ROX concentration (500 nM)

Manufacturer	Model
Applied Biosystems	5700, 7000, 7300, 7700, 7900, 7900HT, 7900HTFast, StepOne, StepOnePlus

Low ROX concentration (50 nM)

Manufacturer	Model
Stratagene	MX4000P, MX3000P, MX3005P
Illumina	Eco Real-Time PCR System
Applied Biosystems	7500, 7500Fast, ViiA 7, QuantStudio

No ROX

Manufacturer	Model
NIPPON Genetics EUROPE	FastGene® qFYR Real-Time PCR System
Bio-Rad	iCycler, CFY96, Chromo4, MJOpticon, Opticon 2, MiniOpticon
Cepheid	SmartCycler
Eppendorf	Mastercycler
QIAGEN	Rotor-Gene Q, Rotor-Gene 3000, Rotor-Gene 6000
Roche	LightCycler 480, LightCycler 2.0
Takara	Thermal Cycler Dice Real Time System

FastGene® Probe One Step Mix with UDG

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 per reaction based on the following table:

Component	20 µl rxn	Final conc.
PCR-grade water	Up to 20 µl	N/A
4x Probe OneStep mix (with UDG)	5 µl	1X
Probe (2-4 µM)	1 µl	200 - 400 nM
Forward primer (2-20 µM)	1 µl	100 - 400 nM
Reverse Primer (2-20 µM)	1 µl	100 - 400 nM
Template RNA	10 ng – 1 µg total RNA	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.
- Place the reaction into the PCR cycler.

Step 3: Run the PCR

- Perform PCR with the following cycling protocol:

Step	Temperature	Duration	Cycles
Reverse Transcription	50 °C	30 min	1
Initial denaturation	95 °C	10 min	1
Denaturation	95 °C	10 sec	30-40
Annealing & Elongation	60 °C	30 sec	
Melt analysis	optional: only when using hybridization probes.		

Contact & Support

NIPPON Genetics EUROPE

Mariaweilerstraße 28 a
52349 Düren
Germany

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

For technical support please contact:
support@nippongenetics.eu