FastGene® IC Green 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 trancsription rather than a 1 hour step using the wild-type enzyme.

Extensive research allowed us to create an intercalating (IC) DNA dye suitable for real-time quantification of amplified DNA without inhibiting the polymerization reaction, often seen with other popular intercalating dyes.

FastGene® IC Green has an optimized buffer mixture, able to efficiently amplify GC- and AT-rich using standard or fast cycling conditions. Unspecific signal detection and lower amplification efficiency originated from primer-dimers are inhibited using small molecule inhibition.

Product Applications

FastGene® IC Green 1-Step RT-qPCR Kit is suited for:

- Gene expression analysis (absolute and relative)
- Detection of low copy genes

Limitation of Use

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

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 is stable 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

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

Related Products

LS47SLRS	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
LS47SHRS	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

Quick Notes

- FastGene® IC Green One Step Mix can replace any commercial Dye based qPCR mixture. The annealing temperature may need to be optimized to account for differences in formulation.
- FastGene® IC Green One Step Mix has a dye which does not inhibit the PCR.
- FastGene® IC Green One Step Mix comes as Lo-ROX or Hi-ROX mix.

Contact & Support

NIPPON Genetics EUROPE
Mariaweilerstraße 28-30
52349 Düren
Germany

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

For technical support please contact:
support@nippongenetics.eu

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Step 1: Prepare the reaction master mix

- Ensure that all reagents are properly thawed and mixed.
- Prepare a reaction 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® IC Green One Step Mix	10 µl	1X
20X FastGene® Scriptase	1 µl	1X
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[1]	1
Denaturation	95 °C	5 sec	40
Annealing & Elongation	60 - 65 °C	20 - 30 sec	
Melt analysis	optional		

[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. The reverse transcriptase will be degraded at this step.

[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.

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™