NIPPON Genetics EUROPE GmbH

FastGene® IC Green 2 x qPCR Universal Mix

Technical Data Sheet

Product Description

NIPPON Genetics EUROPE has developed over the past years extensive knowledge of DNA binding dyes, leading to the development of safe nucleic acid dyes MIDORI^{Green} Advance and MIDORI^{Green} Direct. 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 qPCR Mixes ideally suited for:

- Gene expression analysis (absolute and relativ)
- 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 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

LS40S	FastGene® IC Green 2 x qPCR Universal	20 rxns
LS4001	FastGene® IC Green 2 x qPCR Universal	100 rxns
LS4005	FastGene® IC Green 2 x qPCR Universal	500 rxns
LS4050	FastGene® IC Green 2 x qPCR Universal	5000 rxns
LS41S	FastGene® IC Green 2 x qPCR w/ Fluorescein	20 rxns
LS4101	FastGene® IC Green 2 x qPCR w/ Fluorescein	100 rxns
LS4105	FastGene® IC Green 2 x qPCR w/ Fluorescein	500 rxns
LS4150	FastGene® IC Green 2 x qPCR w/ Fluorescein	5000 rxns

Related Products

LS45S	FastGene® PROBE 2 x qPCR Universal	20 rxns
LS4501	FastGene® PROBE 2 x qPCR Universal	100 rxns
LS4505	FastGene® PROBE 2 x qPCR Universal	500 rxns
LS4550	FastGene® PROBE 2 x qPCR Universal	5000 rxns

Quick Notes

- FastGene® IC Green qPCR 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 has a dye which does not inhibit the PCR.
- FastGene® IC Green comes with separat ROX vial for addition to the master mix.
- FastGene® IC Green w/ Fluorescein comes with 20 nM Fluorescein, which is suitable for the **Biorad iCycler®, MyiQ™ and iQ®5 cyclers.**

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 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® IC Green	10 µl	1X
Forward Primer (10 µM)	0.8 µl	400 nM
Reverse Primer (10 µM)	0.8 µl	400 nM
Template DNA	1 µg genomic DNA 100 ng cDNA	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
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.

[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.

Preparation of ROX

The passive reference dye ROX has been widely used for loading control and volume normalization. Many modern instruments do not need the ROX reference dye at all. The concentration of the reference dye is dependent on the instrument. Please check the instrument's manual for further information. The kit comes with a 50 µM ROX solution, which can be added according to the necessities of the instrument (500 nM for high ROX and 50 nM for low ROX concentration):

Reagent	High ROX	Low ROX
2 x Mix	1 ml	1 ml
50 µM ROX Additive	20 µl	2 µl
ROX concentration / Reaction	500 nM	50 nM

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™

Fluorescein (20 nM)

Manufacturer	Model
Biorad	iCycler®, MyiQ™, iQ®5