

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 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 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 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

| | | |
|---------------|--|-----------|
| LS405 | 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 |
| LS415 | 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 separate 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



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 |