Manual

EconoTaq PLUS GREEN 2X Master Mix

For Research Use Only. Not for use in diagnostic procedures.

IMPORTANT
-20 ºC storage required
immediately upon receipt

BIOSEARCH™ TECHNOLOGIES
GENOMIC ANALYSIS BY LGC

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Contents

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1. Product description

EconoTaq™ PLUS GREEN 2X Master Mix is a ready-to-use PCR master mix containing agarose gel loading buffer and tracking dyes. A PCR reaction is set up simply by combining the EconoTaq PLUS GREEN 2X Master Mix with template DNA, primers and water. After the reaction is complete, it can be loaded directly onto an agarose gel. All necessary reaction components are provided in the Master Mix, which contains the following: 0.1 U/µL of EconoTaq DNA Polymerase, Reaction Buffer (pH 9.0), 400 µM dATP, 400 µM dGTP, 400 µM dCTP, 400 µM dATP, 3 mM $MgCl_2$ and a proprietary mix of PCR Enhancer/Stabilizer and blue and yellow tracking dyes. For difficult templates, such as those containing GC content up to 75%, it has been found that EconoTaq PLUS GREEN 2X Master Mix performs well with denaturation temperatures up to 98 °C.

Tracking dye properties. On a 1% agarose gel, the blue dye migrates at the same rate as a 5 kb DNA fragment, and the yellow dye migrates at 75 bp. The dyes can be removed by standard DNA purification methods, such as binding to an affinity matrix or by ethanol precipitation. EconoTaq PLUS 2X Master Mix with no dyes is also available (Cat. no. 30335-1 or -2). The EconoTaq PLUS GREEN 2X Master Mix is not recommended for any downstream applications using absorbance and fluorescence excitation, as the tracking dyes may interfere with the light detection.

PCR activity: EconoTaq PLUS GREEN 2X Master Mix is tested in DNA amplification using a variety of templates and primers.

Activity determination: One unit of EconoTaq DNA Polymerase catalyses the incorporation of 10 nmoles of dNTP into acid-insoluble material in 30 minutes at 70 °C in 50 mM Tris-HCl (pH 9.0), 50 mM NaCl, 5 mM $MgCl_2$, 200 µM dGTP, dATP, dTTP, dCTP (a mix of unlabeled and [^{33}P]dCTP), 10 µg Activated Calf Thymus DNA and 0.1 mg/mL BSA.

Absence of endonuclease or nicking activity: Incubation of 10 U of EconoTaq DNA Polymerase with 1 µg of supercoiled pBR322 DNA for 16 hours at 70 °C results in no detectable conversion to relaxed or linear forms detectable by agarose gel electrophoresis.

Absence of exonuclease activity: Incubation of 10 U of EconoTaq DNA Polymerase with 1 µg of *Hin*dIII-cut lambda DNA for 16 hours at 70 °C resulted in no smearing of bands on agarose gels.

Purity: EconoTaq DNA Polymerase is >99% pure as determined by SDS-PAGE. There is no detectable DNA contamination.

Deoxynucleotide solution: The nucleotide solutions are certified free of nucleases and phosphatases.

2. Introduction to PCR

The Polymerase Chain Reaction (PCR) is a powerful technique that amplifies specific DNA sequences using multiple cycles of a 3-step process. The first step involves denaturation of a double-stranded DNA template at a high temperature. In the second step, sequence-specific primers anneal to complementary sites flanking the target sequence. In the third step, a thermostable DNA polymerase extends the annealled primers, thereby copying the original DNA sequence. The newly synthesised

DNA becomes the template for subsequent DNA amplification, doubling the amount of template with each cycle. These steps are typically repeated 25-35 times, resulting in a 10^5-10^9 fold increase in the amount of target DNA.

3. Product designations and kit components

Product	Kit size	Catalogue number	Reagent description	Part number	Volume
EconoTaq PLUS GREEN 2X Master Mix*	10 rxns**	30033-0	EconoTaq PLUS Green 2X Master Mix	F93481-0	250 µL
	500 rxns	30033-1	EconoTaq PLUS Green 2X Master Mix	F93481-1	12.5 mL
	1,000 rxns	30033-2	EconoTaq PLUS Green 2X Master Mix	F93481-1	25 mL

* EconoTaq PLUS GREEN 2X Master Mix contains all of the components necessary to perform PCR amplification, except for template and primers. The Master Mix also contains agarose gel loading buffer and tracking dyes.
** Trial size.

4. Storage conditions

EconoTaq PLUS GREEN 2X Master Mix can be stored at -20 °C for 12 months. For everyday use, an aliquot can be stored at +4 °C for up to three months. The EconoTaq PLUS GREEN 2X Master Mix needs to be mixed well prior to use. It is stable for ten freeze-thaw cycles.

5. PCR setup

5.1. **Materials supplied by the user.** PCR amplification is performed by adding template DNA, primers, and water to the EconoTaq PLUS GREEN 2X Master Mix. The following components must be supplied by the user:

- Template DNA (10-50 ng of plasmid DNA; 50-200 ng of genomic DNA)
- Forward primer (100 µM)
- Reverse primer (100 µM)
- Nuclease-free water
- Thermal cycling apparatus

5.2. **Reaction setup.** Set up PCR amplifications of the desired size, according to the following:

				Final concentration
EconoTaq PLUS GREEN 2X Master Mix	12.5 µL	25.0 µL	50.0 µL	1 X
Forward primer (100 µM)	0.25 µL	0.5 µL	1.0 µL	1 µM
Reverse primer (100 µM)	0.25 µL	0.5 µL	1.0 µL	1 µM
DNA template (10 ng/µL)	1.0 µL	1.0 µL	1.0 µL	
Water, nuclease-free	11.0 µL	23.0 µL	47.0 µL	
Total reaction volume	25.0 µL	50.0 µL	100.0 µL	

5.2. Gently mix the PCR components in a thin-walled reaction tube and spin briefly in a microcentrifuge. Add a drop of mineral oil if the thermocycler does not have a heated lid.

6. PCR cycling conditions

6.1. Pre-heat the thermal cycler to 94 °C.

6.2. For initial denaturation of target template DNA, incubate the reactions at 94 °C for two minutes.

6.3. Denature, anneal and extend the DNA according to the following for subsequent cycles of amplification:

Cycling step	Temperature	Time	# of cycles
Initial denaturation*	94 °C	2 min	1
Denaturation*	94 °C	15-30 sec	25-35
Annealing**	50-65 °C	15-30 sec	
Extension	72 °C	1 min/kb	
Final extension	72 °C	5-10 min	1
Hold	4 °C	Indefinitely	1

* EconoTaq PLUS GREEN performs well with denaturation temperatures up to 98 °C.
**Anneal at T_m of primer ± 2 °C. See PCR guidelines section.

6.4. After completion of the PCR, a 5 µL aliquot of the reaction can be loaded directly onto an agarose gel for analysis or size selection. The tracking dyes do NOT interfere with common methods of DNA purification, such as ethanol precipitation or binding to an affinity matrix.

7. PCR guidelines

Optimization of PCR conditions is required for amplification involving templates with high GC content, internal secondary structure or products greater than 5 kb. The following guidelines can be used to improve the success of amplifying these templates:

1) **Cold reaction setup**. Reactions using EconoTaq PLUS GREEN Master Mix yield best results when set up on ice and maintained at 4 °C prior to amplification. The EconoTaq Polymerase has

residual activity at temperatures above 4 °C that can cause non-specific background amplification. Add primers last to reactions just prior to target addition and incubation/cycling.

2) **Template DNA.** Use of purified, high quality template DNA enhances the success rate of PCR. We recommend using 10-50 ng of plasmid DNA and 50-200 ng of genomic DNA. Template should be suspended in water, rather than EDTA-containing solution such as TE buffer.
3) **Primer design.** Oligonucleotide primers for PCR should be 20-25 bases in length and should have a GC content of 40-60%, with the GC bases evenly spaced in the primer. Self-annealling of primers leads to production of primer-dimers, which can diminish the amount of authentic product; therefore, the 3′ end of each primer should not be complementary to itself or to the 3′ end of the opposing primer. The melting temperature (T_m) of the primers should be within 5 °C of each other. The final concentration of each primer in the reaction should be 1 µM.
4) **Annealling.** PCR enhancers in EconoTaq PLUS GREEN Master Mix may lower primer T_m slightly. Optimize the reaction conditions by performing the reaction starting with an annealling temperature 2 °C below the calculated melting temperature of the primers. Vary the annealling temperature to optimise for the primer/template combination. Reducing the annealling temperature improves yield and reaction efficiency, while increasing annealling temperature improves specificity.
5) **Denaturation temperature.** EconoTaq PLUS GREEN 2X Master Mix will amplify most routine DNA templates using a denaturation temperature of 94 °C. For GC-rich templates, with GC content up to 75%, a denaturation temperature of 98 °C has been found to improve results.

8. Technical support and product guarantee

If you require any further support, please do not hesitate to contact our Technical Support Team: techsupport@lgcgroup.com.

Product guarantee: LGC Biosearch Technologies™ guarantees that this product will perform as specified for one year from the date of shipment. Please avoid using reagents for greater than one year from receipt.

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