

EconoTaq[®] PLUS GREEN 2X Master Mix

**IMPORTANT!
-20°C Storage Required
Immediately Upon Receipt**

Lucigen[®] Corporation
Advanced Products for Molecular Biology

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Components & Storage Conditions

Components: 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.

Catalog #	Number of reactions
30033-0 (Trial Size)	10 reactions (1 tube containing 250 µl)
30033-1	500 reactions (10 tubes, each containing 1,250 µl)
30033-2	1000 reactions (20 tubes, each containing 1,250 µl)

Storage: 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.

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 units/µl of EconoTaq DNA Polymerase, Reaction Buffer (pH 9.0), 400 µM dATP, 400 µM dGTP, 400 µM dCTP, 400 µM dTTP, 3 mM MgCl₂, 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 (Lucigen Cat. #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.

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 annealed primers, thereby copying the original DNA sequence. The newly synthesized 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 10⁵-10⁹ fold increase in the amount of target DNA.

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PCR Setup

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 pmol/μl)
Reverse Primer (100 pmol/μl)
Nuclease-free water
Thermocycling apparatus

2) Reaction Setup. Set up PCR amplifications of the desired size, according to the following chart:

	25 μl Reaction	50 μl Reaction	100 μl Reaction	Final concentration
EconoTaq PLUS GREEN 2X Master Mix	12.5 μl	25.0 μl	50.0 μl	1 X
Forward Primer (100 pmol/μl)	0.25 μl	0.5 μl	1.0 μl	1 pmol/μl (1 μM)
Reverse Primer (100 pmol/μl)	0.25 μl	0.5 μl	1.0 μl	1 pmol/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	

3) 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.

PCR Cycling Conditions

- 1) Pre-heat the thermocycler to 94°C.
- 2) For initial denaturation of target template DNA, incubate the reactions at 94°C for two minutes.
- 3) Denature, anneal, and extend the DNA according to the following chart 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 \pm 2°C. See PCR Guidelines section.

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.

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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 Set-Up.** 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-annealing 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 primer concentration in the reaction should be 1 pmol/μl (equal to 1 nmol/ml, or 1 μM).
- 4) **Annealing.** 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 annealing temperature 2°C below the calculated melting temperature of the primers. Vary the annealing temperature to optimize for the primer/template combination. Reducing the annealing temperature improves yield and reaction efficiency, while increasing annealing 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.

Quality Control

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 catalyzes 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 MgCl₂, 200 μM dGTP, dATP, dTTP, dCTP (a mix of unlabeled and [³³P]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 HindIII-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.

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Related Lucigen Products

- EconoTaq[®] PLUS 2X Master Mix
- EconoTaq DNA Polymerase
- TaqSelect[™] DNA Polymerase
- Bst DNA Polymerase, Exonuclease Minus
- PyroPhage[®]3173 DNA Polymerase, Exonuclease Minus (Exo-)
- dNTPs, PCR Grade
- Gel-Ready[™] DNA Ladders
- BigEasy[®] v2.0 Linear Cloning Kit
- CloneSmart[®] Blunt Cloning Kits
- DNATerminator[®] End Repair Kit
- PCRTerminator[®] End Repair Kit
- UltraClone[™] DNA Ligation & Transformation Kits
- CloneDirect[™] Rapid Ligation Kit
- GC Cloning and Amplification Kits
- ClonePlex[®] Library Construction Kit
- pEZSeq[™] Blunt Cloning Kits
- cSMART[™] cDNA Cloning Kits
- *E. coli*[®] EXPRESS Competent Cells
- OverExpress[™] Competent Cells