

Taq98TM Hot Start 2X Master Mix

Optimized for 98C Denaturation

IMPORTANT!
-20°C Storage Required
Immediately Upon Receipt

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Taq98™ Hot Start 2X Master Mix

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Components & Storage Conditions

Components: Taq98™ Hot Start 2X Master Mix contains all of the components necessary to perform PCR amplification, except for template and primers.

Catalog #	Number of reactions
30057-0 (Trial Size)	50 reactions (1 tube containing 1250 µL)
30057-1	250 reactions (5 tubes, each containing 1,250 µL)
30057-2	500 reactions (10 tubes, each containing 1,250 µL)

Storage: Taq98™ Hot Start 2X Master Mix can be stored at -20°C for 12 months. The Taq98™ Hot Start 2X Master Mix needs to be mixed well prior to use. It is stable for ten freeze-thaw cycles.

Description

Taq98™ Hot Start 2X Master Mix is a ready-to-use PCR master mix for 98 °C denaturation. A PCR reaction is set up simply by combining the Taq98™ Hot Start 2X Master Mix with template DNA, primers, and water. All necessary reaction components are provided in the Master Mix, which contains the following as provided: Taq98 DNA Polymerase, Reaction Buffer (pH 8.8, 40 mM Tris), 400 µM dATP, 400 µM dGTP, 400 µM dCTP, 400 µM dTTP, 4 mM MgCl₂, 20 mM KCl, 20 mM (NH₄)₂SO₄, 0.2% Triton X-100, and a proprietary mix of PCR Enhancer/Stabilizer. PCR using Taq98™ 2X Master Mix should undergo denaturation at 98°C for best results.

Introduction to Taq98™ 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 double-stranded DNA templates at the *elevated temperature of 98°C*. In the second step, sequence-specific primers anneal to complementary sites flanking the target sequence. In the third step, thermostable Taq98™ DNA polymerase extends the annealed primers, 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 can be repeated 25-35 times, resulting in a 10⁵-10⁹ fold increase in the amount of target DNA. Additional cycles up to 45 can improve sensitivity but may lead to increased non-specific amplification.

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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 Taq98 Hot Start 2X Master Mix. The following components must be supplied by the user:

Template DNA (10 pg - 50 ng of plasmid DNA; 10-200 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
Taq98 Hot Start 2X Master Mix	12.5 μL	25.0 μL	50.0 μL	1 X
Forward Primer (50 pmol/μL)	0.25 μL	0.5 μL	1.0 μL	0.5 pmol/μL (0.5 μM)
Reverse Primer (50 pmol/μL)	0.25 μL	0.5 μL	1.0 μL	0.5 pmol/μL (0.5 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*	

*DNA may comprise up to 12, 24 or 48 μL of the total volume respectively; use Nuclease-free water to bring to volume.

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 with 98°C Denaturation

- 1) Pre-heat the thermocycler to 98°C (optional).
- 2) For initial denaturation of target template DNA, incubate the reactions at 98°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
Activation / Denaturation*	98°C	2 min	1
Denaturation*	98°C	15 - 30 sec	25 – 40+
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

* Taq98™ Hot Start reactions must undergo denaturation at 98°C for optimal results.

** Anneal at primer melting temperature (T_m) ±2°C. See PCR Guidelines section.

4) After completion of the PCR, analyze by agarose gel electrophoresis.

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Taq98™ PCR Guidelines

Optimization of PCR conditions may be required for amplification of templates with high GC content, internal secondary structure, or products greater than 5 kb. The following guidelines can be used:

- 1) **Reaction Set-Up** Although the Taq98™ master mix has virtually no activity at room temperature, for best results set up and maintain Taq98™ Hot Start reactions at room temperature for no more than 15 minutes prior to amplification. Benchtop incubations of over an hour can cause non-specific background amplification.
- 2) **Template DNA.** Use purified, high quality template DNA to enhance the success rate of PCR. We recommend using 10 pg - 50 ng of plasmid DNA and 10 - 200 ng of genomic DNA. Template should be dissolved in water, rather than EDTA-containing solution such as TE buffer. The amount of template DNA required is dependent on several factors, including the size and complexity of the template DNA and the number of PCR cycles.
- 3) **Primer Design.** Oligonucleotide primers for PCR are generally 20-25 bases in length and 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. The 3' end of each primer should not be complementary to any portion of either itself or the opposing primer. The melting temperature of the primers should be within 5°C of each other. The final primer concentration in the reaction should be 0.2-1.0 uM
- 4) **Annealing.** Taq98™ Hot Start 2X Master Mix contains PCR enhancers that may lower primer T_m slightly. Set the annealing temperature to 2°C below the calculated melting temperature of the primers. If necessary, optimize the annealing temperature for the primer/template combination. Reducing the annealing temperature improves yield and reaction efficiency, while increasing annealing temperature improves specificity.
- 5) **Denaturation Temperature.** Taq98™ master mix has been designed to undergo a 2-minute denaturation at 98 °C to enable Taq98 to amplify the very toughest templates such as those with high GC content or challenging secondary structure.

Quality Control

PCR Activity: Taq98™ Hot Start 2X Master Mix is tested in DNA amplification using a variety of templates and primers.

Activity Determination: One unit of enzyme catalyzes the incorporation of 10 nmoles of dNTP into acid-insoluble material in 10 minutes at 70°C in 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1.5 mM MgCl₂, 200 µM dGTP, dATP, dTTP, dCTP (a mix of unlabeled and [33P]dCTP), 10 µg Activated Calf Thymus DNA, 0.1% Triton X-100 and 0.1 mg/ml BSA.

Absence of Endonuclease or Nicking Activity: Incubation of 10 U of Taq98 DNA Polymerase with 1 µg of supercoiled pUC19 DNA for 16 hours at 37°C results in no detectable conversion to relaxed or linear forms detectable by agarose gel electrophoresis.

Purity: Taq98 DNA Polymerase is >95% pure as determined by SDS-PAGE. There is no detectable DNA contamination.

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Related Lucigen Products

- EconoTaq® PLUS Hot Start 2X Master Mix
- EconoTaq® PLUS GREEN Hot Start 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
- DNATerminator® End Repair Kit
- PCRTerminator® End Repair Kit
- CloneDirect™ Rapid Ligation Kit
- GC Cloning and Amplification Kits
- ClonePlex® Library Construction Kit
- pEZSeq™ Blunt Cloning Kits
- cSMART™ cDNA Cloning Kits
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