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AuPreP Family

AuPreP Citations

AuPreP™ DIAMOND 2x DOUBLE DYE MASTER-MIX

A high density 2X Master Mix containing ultrapure and highly sensitive Taq DNA polymerase, dNTPs, PCR enhancer & stabilizer, buffer with the magnesium concentration optimal for routine PCR, gel loading & tracking dyes.

Components: AuPreP DIAMOND 2X DOUBLE DYE 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 # AUP-DMX-50 AuPreP DIAMOND 2X DOUBLE DYE MASTER-MIX 50 Rx
(1 tube containing 1250µl)

Storage: AuPreP DIAMOND 2X DOUBLE DYE 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. AuPreP DIAMOND 2X DOUBLE DYE MASTER-MIX needs to be mixed well prior to use. It is stable for ten freeze-thaw cycles.

Product Description : AuPreP DIAMOND 2X DOUBLE DYE 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 AuPreP DIAMOND 2X DOUBLE DYE 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 AuPreP DIAMOND 2X DOUBLE DYE MASTER-MIX, which contains the following: 0.1 units/µl of ultrapure & highly sensitive Taq DNA Polymerase, Reaction Buffer (pH 9.0), 400 µM dATP, 400 µM dGTP, 400 µM dCTP, 400 µM dTTP, 3 mM MgCl₂, and a supermix of PCR Enhancer/Stabilizer and blue and yellow Tracking Dyes.

Tracking Dye related Features: 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. AuPreP DIAMOND 2X MASTER-MIX with no dyes is also available. AuPreP DIAMOND 2X DOUBLE DYE MASTER-MIX is not advised for any downstream applications using absorbance and fluorescence excitation, as the tracking dyes may interfere with the light detection.

Quality Features & Controls

PCR Activity: AuPreP DIAMOND 2X DOUBLE DYE MASTER-MIX is tested in DNA amplification using a variety of templates and primers.

Activity Determination: One unit of Taq 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 Taq 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 Taq DNA Polymerase with 1 µg of Hind III-cut lambda DNA for 16 hours at 70°C resulted in no smearing of bands on agarose gels.

Purity: Taq DNA Polymerase is ultrapure and highly sensitive. It is >99% pure as determined by SDS PAGE with no detectable DNA contamination.



Deoxynucleotide Solution: The nucleotide solns. are ultrapure, certified free of nucleases & phosphatases.

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 10^5 - 10^9 fold increase in the amount of target DNA.

PCR Setup

1) Materials supplied by the user. PCR amplification is performed by adding template DNA, primers, and water to the AuPreP DIAMOND 2X DOUBLE DYE 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/ μ l)
- Reverse Primer (100 pmol/ μ l)
- Nuclease-free water
- Thermocycling apparatus

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

	25uL	50uL	100uL	final conc
AuPreP DIAMOND 2X DOUBLE DYE MASTER-MIX	12.5 μ l	25.0 μ l	50.0 μ l	1 X
Forward Primer (100 pmol/ μ l)	0.25 μ l	0.5 μ l	1.0 μ l	1 pmol/ μ l
Reverse Primer (100 pmol/ μ l)	0.25 μ l	0.5 μ l	1.0 μ l	1 pmol/ μ l
DNA template (10 ng/ μ l)	1.0 μ l	1.0 μ l	1.0 μ l	< 50 ng
Water, Nuclease-free	11.0 μ l	23.0 μ l	47.0 μ l	---

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

*Anneal at T_m of primer \pm 2°C.



PCR Cycling Conditions (cont'd)-

4) After completion of the PCR, a 5 μ L 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.

AuPreP DIAMOND 2x DOUBLE DYE MASTER-MIX 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:

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.

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/ μ l (equal to 1 nmol/ml, or 1 μ M).

<u>Other AuPreP™ DNA/RNA Kits</u>	<u>Other Related Products</u>
AuPreP™ Plasmid Maxi Kit	AuPreP Oligos (High Affinity Purified Oligo synthesis available in different scales, purifications & modifications)
AuPreP™ Plasmid Midi Kit	AuPreP TaQ DNA Polymerase (Ultrapure, Ultra-stable & Ultra-sensitive Taq DNA Polymerase)
AuPreP™ SPIN™ SPIN Miniprep Kit	AuPreP Hotstart TaQ DNA Polymerase (Robust Polymerase for Hotstart PCR assays)
AuPreP™ Blood Genomic DNA Maxi	AuPreP Super Fidelity TaQ DNA Polymerase (High fidelity Polymerase produces blunt ended amplicons upto 5Kb)
AuPreP™ Blood Genomic DNA Extraction Midi Kit	PCR Doctor - (PCR enhancer for AuPreP Hotstart Taq or Super Fidelity Taq especially designed for GC/AT/Dirty/Difficult Templates)
AuPreP™ GEN ^{ht} DNA Extraction Kit	AuPreP Longjump Polymerase (Robust Long Polymerase for templates > 4kb to 18kb+ for challenging PCRs)
AuPreP™ DNA easy Plant Maxi kit	AuPreP Red PCR Master Mix (2x Master mix with Red Dye without Enhancer)
AuPreP™ DNA easy Plant Mini Kit	AuPreP DIAMOND MASTER-MIX (2x Mastermix with PCR Enhancer & Stabilizer without tracking dyes)
AuPreP™ PCR Purification Kit	AuPreP DIAMOND DOUBLE DYE MASTERMIX (2x Mastermix with PCR Enhancer, Stabilizer & tracking dyes)
AuPreP™ Plant RNA Maxi Kit	AuPreP DNA Extraction System (A fast Reagent for pure genomic DNA isolation for down stream applications)
AuPreP™ Plasmid Maxi Kit	AuPreP RNA Extraction System (for Purest & High Quality RNA extraction with simple cost effective protocol)
AuPreP™ RNA Easy Midi Kit	AuPreP Gold cDNA Synthesis Kit (Highly Cost effective cDNA Synthesis Kit using RT with reduce Rnase H activity)
	AuPreP Gold RT-PCR Combo Kit (2 step RT-PCR protocol with tracking Dye)
	AuPreP Extra Mile First Strand cDNA System (Premium cDNA Synthesis Kit using RT with point mutant Rnase H minus activity)



AuPreP™ RNA™ Mini Kit	Novascript III RNase H⁻ RT (Premium Ultra-stable Rnase H minus RT for long high quality cDNA construction)
AuPreP™ RNV™ Viral RNA Extraction Miniprep Kit	Novascript III single step RT-PCR System (Premium 1step RT-PCR system using Novascript & AuPreP Hotstart DNA Polymerase) AuPreP Random Primer labeling Mix System (Premixed solution for the labeling of DNA with radiolabeled dCTP using random sequence oligonucleotides)

For more information and ordering please contact:

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