

TaqSelect™ DNA Polymerase 2X Premix Without dNTPs

IMPORTANT!
-20°C Storage Required
Immediately Upon Receipt

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Advanced Products for Molecular Biology

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TaqSelect™ DNA Polymerase 2X Premix Without dNTPs

Components & Storage Conditions

Components: TaqSelect™ DNA Polymerase 2X Premix contains all of the components necessary to perform PCR amplification, except for template, primers and dNTPs (deoxynucleotides).

This premix does not contain dNTPs (deoxynucleotides).

Catalog #	Number of reactions
30041-0	10 reactions (1 tube containing 250µl, Trial Size)
30041-1	500 reactions (10 tubes, each containing 1,250µl)
30042-2	1000 reactions (20 tubes, each containing 1,250µl)

Storage: TaqSelect™ DNA Polymerase 2X Premix 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 TaqSelect™ DNA Polymerase 2X Premix needs to be mixed by vortexing vigorously prior to use. It is stable for ten freeze-thaw cycles.

Description

TaqSelect™ DNA Polymerase 2X Premix is a ready-to-use PCR mix without dNTPs. Just as with other Taq polymerases, the amplification products of TaqSelect DNA Polymerase product will have an additional A or G overhang and can be cloned into GC Cloning vectors or TA cloning vectors. For difficult templates, such as those containing GC content up to 75%, it has been found that TaqSelect DNA Polymerase performs well with denaturation temperatures up to 98°C. This Premix does not contain dNTPs (deoxynucleotides). PCR grade dNTPs, can be purchased separately from Lucigen (Cat. No. 30030-1 is a mixture of 2.5 mM of each dNTP and is conveniently formulated for use with this protocol). A PCR reaction is set up simply by combining the TaqSelect™ DNA Polymerase 2X Premix with template DNA, primers, dNTPs, and water. All other necessary components are provided in the 2X Premix, which contains the following: 0.1 units/µl of Taq DNA Polymerase, Reaction Buffer (pH 9.0), 3 mM MgCl₂, and a proprietary PCR Enhancer/Stabilizer.

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, dNTPs, and water to the TaqSelect™ DNA Polymerase 2X Premix. 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)
- dNTP mixture (deoxynucleotides– 2.5 mM each, available separately from Lucigen; Cat. No. 30030-1)
- Nuclease-free water
- Thermocycling apparatus

2) Reaction Setup. Set up PCR amplifications of the desired size (on ice for the best results), according to the following chart: **(Note: This premix does not contain dNTPs).**

	25 μl Reaction	50 μl Reaction	100 μl Reaction	Final concentration
TaqSelect™ 2X Premix	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
Reverse Primer (100 pmol/ μl)	0.25 μl	0.5 μl	1.0 μl	1 pmol/ μl
DNA template (10 ng/ μl)	1.0 μl	1.0 μl	1.0 μl	
dNTP mixture (2.5 mM each)	2.0 μl	4.0 μl	8.0 μl	0.2 mM each
Water, Nuclease-free	9.0 μl	19.0 μl	39.0 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

- Pre-heat the thermocycler to 94°C.
- For initial denaturation of target template DNA, incubate the reactions at 94°C for two minutes.
- 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

* TaqSelect DNA Polymerase performs well with denaturation temperatures up to 98°C.

**Anneal at T_m of primer ± 2°C. See PCR Guidelines section.

4) After completion of the PCR, a 5-μl aliquot of the reaction is loaded onto an agarose gel for analysis or size selection.

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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 TaqSelect DNA Polymerase yield best results when set up on ice and maintained at 4°C prior to amplification. The TaqSelect 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/ μ l (equal to 1 nmol/ml, or 1 μ M).
- 4) **Annealing.** PCR enhancers in TaqSelect DNA Polymerase 2X Premix 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.** TaqSelect DNA Polymerase 2X Premix 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: TaqSelect™ DNA Polymerase 2X Premix is tested in DNA amplification using a variety of templates and primers.

Activity Determination: One unit of TaqSelect™ 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 μ M dGTP, dATP, dTTP, dCTP (a mix of unlabeled and [³³P]dCTP), 10 μ g Activated Calf Thymus DNA, and 0.1 mg/ml BSA.

Absence of Endonuclease or Nicking Activity: Incubation of 10 U of TaqSelect™ DNA Polymerase with 1 μ g 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 TaqSelect™ DNA Polymerase with 1 μ g of HindIII-cut lambda DNA for 16 hours at 70°C resulted in no smearing of bands on agarose gels.

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

Related Lucigen Products

- dNTPs, PCR Grade
- EconoTaq® PLUS and PLUS GREEN 2X Master Mixes
- EconoTaq DNA Polymerase
- Bst DNA Polymerase, Exonuclease Minus
- PyroPhage® 3173 DNA Polymerase, Exonuclease Minus (Exo-)
- 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