

NovaTaq™ DNA Polymerase and Kits

About the Kits

NovaTaq™ DNA Polymerase	100 U	71003-3
	500 U	71003-4
	2500 U	71003-5
NovaTaq PCR Master Mix	200 rxn	71007-3

Description

NovaTaq™ DNA Polymerase is a premium quality recombinant form of *Thermus aquaticus* DNA polymerase. The enzyme possesses 5'→3' DNA polymerase activity and lacks 3'→5' exonuclease activity. The preparation is >95% homogenous by SDS-PAGE and lacks detectable endonuclease activities. NovaTaq is prequalified for use in standard PCR amplification protocols and is extensively tested in a variety of quality control assays. NovaTaq generates PCR products with 3'-dA overhangs, suitable for cloning with Perfectly Blunt®, AccepTor™, and LIC Vector Kits.

NovaTaq DNA Polymerase is also available in three complete kits. The NovaTaq PCR Kit contains all reagents necessary for PCR amplification except primers and template. The NovaTaq PCR Kit PLUS includes 1.5 ml of 10X NovaTaq Optimization Buffer in addition to all of the NovaTaq PCR Kit reagents. The NovaTaq Optimization Buffer offers advantages of greater tolerance to variable Mg²⁺ concentrations and a wider temperature window for optimal primer:template annealing. The NovaTaq PCR Master Mix is a ready-to-use 2X concentrated mixture of NovaTaq DNA Polymerase, ultrapure deoxynucleotides, and reaction buffer without MgCl₂. Simply add the NovaTaq PCR Master Mix to an equal volume containing the required amount of MgCl₂, DNA, template, and primers, and the reaction is ready for thermal cycling.

NovaTaq Unit definition: One unit is defined as the amount of enzyme that will catalyze the incorporation of 10 nmol of dNTP into acid-insoluble form in 30 min at 74°C in a reaction containing 25 mM TAPS (tris-[hydroxymethyl]-methyl-amino-propane-sulfonic acid, sodium salt), pH 9.3 at 25°C, 50 mM KCl, 2 mM MgCl₂, 1 mM β-mercaptoethanol, 0.2 mM dATP, dGTP, and dTTP, 0.1 μM [α-³²P] dCTP, and activated salmon sperm DNA.

© 2011 EMD Chemicals, Inc., an affiliate of Merck KGaA, Darmstadt, Germany. All rights reserved. The Novagen name and logo, and Perfectly Blunt® are registered trademarks of Merck KGaA, Darmstadt, Germany. AccepTor™ and NovaTaq™ are trademarks of Merck KGaA, Darmstadt, Germany. Triton® is a registered trademark of Dow Chemical Company.

Use of this product is covered by one or more of the following US patents and corresponding patent claims outside the US: 5,789,224, 5,618,711, 6,127,155 and claims outside the US corresponding to expired US Patent 5,079,352. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim, no right to perform any patented method, and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel. This product is for research use only. Diagnostic uses under Roche patents require a separate license from Roche. Further information on purchasing licenses may be obtained by contacting the Director of Licensing, Applied Biosystems, 850 Lincoln Centre Drive, Foster City, California, 94404, USA.

USA and Canada

Tel (800) 628-8470
bioscienceshelp@
emdchemicals.com

Europe

France Freephone 0800 126 461	Germany Freecall 0800 100 3496	Ireland Toll Free 1800 409 445	United Kingdom Freephone 0800 622 935	All other European Countries +44 115 943 0840
--	---	---	--	---

All Other Countries

Contact Your Local Distributor
www.merck4biosciences.com
bioscienceshelp@
emdchemicals.com

techservice@merckbio.eu

www.merck4biosciences.com

FOR RESEARCH USE ONLY. NOT FOR HUMAN OR DIAGNOSTIC USE.

Components

NovaTaq™ DNA Polymerase

- 100 U or 500 U or 5 × 500 U NovaTaq DNA Polymerase (5 U/μl)
- 1 × or 2 × or 7 × 1.5 ml 10X NovaTaq Buffer with MgCl₂ (10X = 500 mM KCl, 100 mM Tris-HCl pH 9.0 at 25°C, 15 mM MgCl₂, 1% Triton® X-100)
- 1 × or 2 × or 7 × 1.5 ml 10X NovaTaq Buffer without MgCl₂ (10X = 500 mM KCl, 100 mM Tris-HCl pH 9.0 at 25°C, 1% Triton X-100)
- 1 × or 2 × or 7 × 1.5 ml 25 mM MgCl₂

NovaTaq PCR Master Mix

- 4 × 1.25 ml NovaTaq PCR Master Mix (includes 250 U NovaTaq DNA Polymerase)
- 3 × 2 ml PCR Grade Water
- 1.5 ml 25 mM MgCl₂

Storage

Store all components at -20°C.

Standard Protocols

The standard PCR protocol described below will provide satisfactory amplification in many cases. Remember to include a negative control reaction lacking only template as well as a positive control reaction using a template known to amplify with the primers. A colony PCR protocol for screening bacterial colonies is also included below. Note, the optimal conditions for the concentrations of enzyme, MgCl₂, template, and primers may need to be determined empirically.

Standard PCR with NovaTaq DNA Polymerase

1. For each 50 μl reaction, assemble the following in a 0.5 ml PCR tube on ice just prior to use:

40.75 μl	PCR Grade Water
1 μl	dNTP Mix (10 mM each dATP, dCTP, dGTP, dTTP)
1 μl	5' primer, ~5 pmol/μl
1 μl	3' primer, ~5 pmol/μl
5 μl	10X NovaTaq Buffer with MgCl ₂
0.25 μl	NovaTaq DNA Polymerase (1.25 U)
1 μl	DNA template (typically 10 ng)
50 μl	Total volume

Note: If using NovaTaq Buffer without MgCl₂, add 25 mM MgCl₂ to a final concentration of 1.5–2.5 mM and decrease the volume of water added to the reaction to compensate.

2. Mix gently. If necessary, centrifuge briefly. Cap tubes and place in thermal cycler.
3. Process in thermal cycler for 30 cycles as follows:
 - Denature 1 min at 94°C
 - Anneal 1 min at the proper annealing temperature
 - Extend 2 min at 72°C
 - Repeat for 30 cycles
 - Final extension for 5 min at 72°C

Note: Optimal conditions for amplification will vary depending on the primers and thermal cycler used. It may be necessary to optimize the system for individual primers, template, and thermal cycler.
4. If necessary, polymerase can be inactivated by chloroform extraction. Add 100 μl chloroform, mix for 30 s, and centrifuge for 1 min. The top aqueous phase (which may appear cloudy) contains the DNA products. Transfer aqueous phase to fresh tube.
5. Load and run a 1% agarose gel containing 0.5 μg/ml ethidium bromide. Visualize bands under UV illumination.

Colony PCR

- Pick a colony from an agar plate using 200 μ l pipet tip or sterile toothpick. Choose colonies that are at least 1 mm in diameter, and try to collect as many cells as possible. If a copy of the colony is desired, touch pipet tip to a fresh plate, before transferring.
- Transfer bacteria to 0.5 ml tube containing 50 μ l sterile water. Vortex to disperse cells.
- Place tubes in boiling water or heat block at 99°C for 5 min to lyse cells and denature DNases.
- Centrifuge at 12,000 \times g for 1 min to remove cell debris.
- Transfer 10 μ l supernatant to fresh 0.5 ml tube for PCR. Leave on ice until use.
- Prepare a master reaction mix by assembling the following components on ice. To account for pipetting losses, it is convenient to multiply the amounts by X .5, where X is the number of reactions. For each reaction, combine:

31.75 μ l	PCR Grade Water
1 μ l	dNTP Mix (10 mM each dATP, dCTP, dGTP, dTTP)
1 μ l	5' primer, ~5 pmol/ μ l
1 μ l	3' primer, ~5 pmol/ μ l
5 μ l	10X NovaTaq Buffer with MgCl ₂
0.25 μ l	NovaTaq™ DNA Polymerase (1.25 U)
40 μ l	Total volume

Note: If using NovaTaq™ Buffer without MgCl₂, add 25 mM MgCl₂ to a final concentration of 1.5–2.5 mM and decrease the volume of water added to the reaction to compensate.
- Mix gently. If necessary, centrifuge briefly. Add 40 μ l master mix to each 10 μ l sample. Mix gently and cap tubes. Place tubes in thermal cycler.
- Process in thermal cycler for 35 cycles as follows:

Denature	1 min at 94°C
Anneal	1 min at the proper annealing temp (usually 55°C for vector primers)
Extend	2 min at 72°C
Repeat for 35 cycles	
Final extension	for 6 min at 72°C
- If necessary, polymerase can be inactivated by chloroform extraction. Add 100 μ l chloroform, mix 30 s, and centrifuge for 1 min. The top aqueous phase (which may appear cloudy) contains the DNA products. Transfer aqueous phase to fresh tube.
- Load and run a 1% agarose gel containing 0.5 μ g/ml ethidium bromide. Visualize bands under UV illumination.

Alternatives

A popular alternative to the standard PCR protocol is the manual hot start procedure. In this procedure, the template is denatured and maintained at 80–94°C before adding the NovaTaq DNA Polymerase.

Adjust the standard PCR protocol as follows: Initiate the cycling procedure with a 94°C step for 1 min followed by a hold step at 80°C. Add enzyme to each reaction tube and mix well. Proceed with the standard cycling conditions.

Adjust the colony PCR protocol as follows: Place 10 μ l of the denatured colony in a 0.5 ml tube (step 5, “Colony PCR”). Incubate in the thermal cycler at 80°C for 1–2 min, and then add 40 μ l of master mix with enzyme. Overlay with oil and proceed with the standard cycling conditions.

NovaTaq PCR Master Mix

The NovaTaq PCR Master Mix is a 2X mixture of NovaTaq DNA Polymerase, dNTPs and NovaTaq Buffer without MgCl₂. When using NovaTaq PCR Master Mix, assemble the components of each 50 μ l reaction on ice as follows:

25 μ l	NovaTaq PCR Master Mix
1 μ l	5' primer, ~5 pmol/ μ l
1 μ l	3' primer, ~5 pmol/ μ l
X μ l	PCR Grade Water (to final volume of 50 μ l)
1 μ l	DNA template (typically 10 ng)
3–5 μ l	25 mM MgCl ₂ (final concentration of 1.5–2.5 mM)
50 μ l	Total volume

Proceed with the standard cycling conditions outlined under “Standard PCR with NovaTaq DNA Polymerase” above (step 3).