Nova*Taq*™ DNA Polymerase and Kits

About the Kits

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

Description

Nova*Taq*™ 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. Nova*Taq* is prequalified for use in standard PCR amplification protocols and is extensively tested in a variety of quality control assays. Nova*Taq* generates PCR products with 3'-dA overhangs, suitable for cloning with Perfectly Blunt®, **A**ccep**T**or™, and LIC Vector Kits.

Nova*Taq* DNA Polymerase is also available in three complete kits. The Nova*Taq* PCR Kit contains all reagents necessary for PCR amplification except primers and template. The Nova*Taq* PCR Kit PLUS includes 1.5 ml of 10X Nova*Taq* Optimization Buffer in addition to all of the Nova*Taq* PCR Kit reagents. The Nova*Taq* Optimization Buffer offers advantages of greater tolerance to variable Mg^{2+} concentrations and a wider temperature window for optimal primer:template annealing. The Nova*Taq* PCR Master Mix is a ready-to-use 2X concentrated mixture of Nova*Taq* DNA Polymerase, ultrapure deoxynucleotides, and reaction buffer without $MgCl_2$. Simply add the Nova*Taq* 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.

Nova*Taq* 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 $MgCl_2$, 1 mM β-mercaptoethanol, 0.2 mM dATP, dGTP, and dTTP, 0.1 µM [α-^{32}P] dCTP, and activated salmon sperm DNA.

Components

Nova*Taq*™ DNA Polymerase

- 100 U or 500 U or 5 × 500 U Nova*Taq* DNA Polymerase (5 U/µl)
- 1 × or 2 × or 7 × 1.5 ml 10X Nova*Taq* Buffer with $MgCl_2$ (10X = 500 mM KCl, 100 mM Tris-HCl pH 9.0 at 25°C, 15 mM $MgCl_2$, 1% Triton® X-100)
- 1 × or 2 × or 7 × 1.5 ml 10X Nova*Taq* Buffer without $MgCl_2$ (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 $MgCl_2$

Nova*Taq* PCR Master Mix

- 4 × 1.25 ml Nova*Taq* PCR Master Mix (includes 250 U Nova*Taq* DNA Polymerase)
- 3 × 2 ml PCR Grade Water
- 1.5 ml 25 mM $MgCl_2$

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, $MgCl_2$, template, and primers may need to be determined empirically.

Standard PCR with Nova*Taq* 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 Nova*Taq* Buffer with $MgCl_2$
0.25 µl	Nova*Taq* DNA Polymerase (1.25 U)
1 µl	DNA template (typically 10 ng)
50 µl	Total volume

   *Note: If using Nova*Taq *Buffer without $MgCl_2$, add 25 mM $MgCl_2$ 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

1. Pick a colony from an agar plate using 200 µl 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.
2. Transfer bacteria to 0.5 ml tube containing 50 µl sterile water. Vortex to disperse cells.
3. Place tubes in boiling water or heat block at 99°C for 5 min to lyse cells and denature DNases.
4. Centrifuge at 12,000 × g for 1 min to remove cell debris.
5. Transfer 10 µl supernatant to fresh 0.5 ml tube for PCR. Leave on ice until use.
6. 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 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 Nova*Taq* Buffer with $MgCl_2$
0.25 µl	Nova*Taq*™ DNA Polymerase (1.25 U)
40 µl	Total volume

   *Note: If using Nova*Taq™ *Buffer without $MgCl_2$, add 25 mM $MgCl_2$ to a final concentration of 1.5–2.5 mM and decrease the volume of water added to the reaction to compensate.*

7. Mix gently. If necessary, centrifuge briefly. Add 40 µl master mix to each 10 µl sample. Mix gently and cap tubes. Place tubes in thermal cycler.
8. 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	

9. If necessary, polymerase can be inactivated by chloroform extraction. Add 100 µl 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.
10. Load and run a 1% agarose gel containing 0.5 µg/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 Nova*Taq* 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 µl 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 µl of master mix with enzyme. Overlay with oil and proceed with the standard cycling conditions.

Nova*Taq* PCR Master Mix

The Nova*Taq* PCR Master Mix is a 2X mixture of Nova*Taq* DNA Polymerase, dNTPs and Nova*Taq* Buffer without $MgCl_2$. When using Nova*Taq* PCR Master Mix, assemble the components of each 50 µl reaction on ice as follows:

25 µl	Nova*Taq* PCR Master Mix
1 µl	5' primer, ~5 pmol/µl
1 µl	3' primer, ~5 pmol/µl
X µl	PCR Grade Water (to final volume of 50 µl)
1 µl	DNA template (typically 10 ng)
3–5 µl	25 mM $MgCl_2$ (final concentration of 1.5–2.5 mM)
50 µl	Total volume

Proceed with the standard cycling conditions outlined under "Standard PCR with Nova*Taq* DNA Polymerase" above (step 3).