NovaTaq™ Hot Start DNA Polymerase Kits

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Description

Nova*Taq* Hot Start DNA Polymerase is a chemically modified form of *Taq* DNA Polymerase that is inactive at room temperature. The 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 enzyme preparation is > 95% homogenous by SDS-PAGE and lacks detectable RNase and endonuclease activities. The enzyme provides improved specificity when compared to standard *Taq* polymerase and can eliminate the presence of nonspecific amplification such as primer-dimers and misprimed products. The enzyme must be activated by heat treatment (7–10 min at 95°C), after which thermal cycling can proceed. Nova*Taq* Hot Start DNA Polymerase generates PCR products with 3'-dA overhangs, suitable for cloning with Novagen® Perfectly Blunt®, **A**ccep**T**or™, and LIC Vector Kits.

Nova*Taq* Hot Start Master Mix Kit is a ready-to-use 2X mixture of Nova*Taq* Hot Start DNA Polymerase, ultrapure deoxynucleotides, and reaction buffer with $MgCl_2$. The Master Mix simplifies the assembly of PCR reactions and offers advantages of time savings, consistency, and minimal risk of contamination. In addition to the Master Mix, the kit includes PCR Grade Water and $MgCl_2$ for increasing Mg^{2+} concentrations. Simply add the Nova*Taq* Hot Start Master Mix to an equal volume containing DNA template, primers, and, if desired, additional $MgCl_2$. The final diluted reaction contains 1.25 U of Nova*Taq* Hot Start DNA Polymerase per 50 µl. The two Nova*Taq* Hot Start Master Mix Kit sizes provide sufficient components for 200 or 1000 standard 50 µl reactions.

Nova*Taq* Hot Start 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 72°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 12.5 µg activated salmon sperm DNA in a 50 µl volume.

Components

Nova*Taq*™ Hot Start DNA Polymerase

- 1 x or 5 x 250 U — Nova*Taq* Hot Start DNA Polymerase (5 U/µl)
- 1 x or 5 x 1.5 ml — 10X NovaTaq Hot Start Buffer (10X = 670 mM Tris-HCl, 160 mM $(NH4)_2SO_4$, 0.1% TWEEN®-20)
- 1 x or 5 x 1.5 ml — 25 mM $MgCl_2$

Nova*Taq* Hot Start Master Mix Kit

- 4 x or 20 x 1.25 ml — NovaTaq Hot Start Master Mix (includes 250 U NovaTaq Hot Start DNA Polymerase)
- 3 x or 11 x 2 ml — PCR Grade Water
- 1 x or 3 x 1.5 ml — 25 mM $MgCl_2$

Storage

Store all components in a constant-temperature freezer at –20°C.

Standard Protocols

This standard PCR protocol will provide satisfactory amplification in many cases. Include a negative control reaction lacking a template, and a positive control reaction using a template known to amplify with the primers. The optimal concentrations of enzyme, $MgCl_2$, template, and primers are determined empirically.

Standard PCR with Nova*Taq* Hot Start DNA Polymerase

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

X µl	PCR Grade Water (to final volume of 50 µl)
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* Hot Start Buffer
3–5 µl	25 mM $MgCl_2$
0.25 µl	Nova*Taq* Hot Start DNA Polymerase (1.25 U)
1 µl	DNA template (typically 10–100 ng)
50 µl	total volume

2. Mix gently. If necessary, centrifuge briefly. Add 2 drops mineral oil (if appropriate). Cap tubes. Place tubes in thermal cycler.
3. Activate the NovaTaq Hot Start DNA Polymerase with a 7–10 min 95°C incubation followed by the following cycling program (or other optimized cycling program):

Denature	30 s at 94°C
Anneal	30 s at the proper annealing temperature
Extend	1 min at 72°C

   Repeat for 30 cycles
   Final extension for 10 min at 72°C

   Note: Any cycling program can be used with NovaTaq Hot Start DNA Polymerase with the addition of the polymerase activation step for 7–10 min at 95°C.

4. To remove oil overlay and inactivate polymerase, 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. Load and run a 1% agarose gel containing 0.5 µg/ml ethidium bromide. Visualize bands under UV illumination.

Using NovaTaq™ Hot Start Master Mix

The Nova*Taq* Hot Start Master Mix is a 2X mixture of Nova*Taq* Hot Start DNA Polymerase, dNTPs and Nova*Taq* Buffer with $MgCl_2$. When using Nova*Taq* Hot Start Master Mix, assemble the components of each 50 µl reaction as follows:

25 µl	Nova*Taq* Hot Start 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)
Y µl	25 mM $MgCl_2$ (if desired, see note below)
50 µl	total volume

Proceed with the standard cycling conditions as outlined in the previous section.

Note: The reaction conditions above will contain a final concentration of 1.5 mM $MgCl_2$. For some applications, increased $MgCl_2$ concentrations may be optimal. Table 1 below shows the volume of 25 mM $MgCl_2$ to be added to each 50 µl reaction to achieve the desired final concentration.

Final $MgCl_2$ Concentration	Volume of 25 mM $MgCl_2$ to add per 50 µl reaction
1.5 mM	0 µl
2.0 mM	1 µl
2.5 mM	2 µl