



GemTaq™ Hot Start DNA Polymerase and Colored 5xReaction Buffer (with 5x1.5 mM Mg²⁺)

(Catalog #EP042, #EP043, #EP044, #EP045)

FOR LABORATORY USE ONLY

Description

GemTaq™ Hot Start DNA Polymerase is a heat-activated high-performance proprietary formulation of GemTaq™ DNA Polymerase. The modified polymerase activity is restored during the initial denaturation step (94-95 °C for 3 min.) allowing for a hot-start PCR. Therefore GemTaq™ Hot Start DNA Polymerase provides improved specificity and very high PCR sensitivity by eliminating the presence of non-specific products. It has an elongation rate higher than regular *Taq* DNA Polymerase, resulting in robust yields and greater sensitivity of PCR amplifications within a short reaction time. GemTaq™ Hot Start DNA Polymerase has 5'→3' DNA polymerase activity and 5'→3' exonuclease activity, the same fidelity as *Taq* polymerase. The enzyme can amplify fragments of up to 5 kb in length from genomic DNA and longer fragments from Lambda DNA. GemTaq™ Hot Start DNA Polymerase is supplied at 5u/μl and comes with an optimized Colored 5xReaction buffer allowing to load PCR samples directly onto gels for electrophoresis. The purified enzyme has no detectable endonuclease or exonuclease activity.

Advantages

- Ultra-high specificity.
- Fast activation.
- Greater efficiency and sensitivity in a wide range of PCR assays within a short reaction time.
- Recommended for low copy assays.
- Amplifies fragments up to 5 kb.
- Unbeatable value in terms of cost per unit and performance.

Applications

- Hot start PCR.
- High throughput PCR applications
- Low-copy target.
- Complex genomic templates.
- TA cloning.

Unit Definition

One unit is defined as the amount of enzyme that will incorporate 10 nmols of dNTPs into acid-insoluble material in 30 minutes at 72°C under the assay conditions.

Unit Assay Conditions

The polymerase activity was assayed in 25 mM TAPS (pH 9.3 at 25°C); 50 mM KCl; 2 mM MgCl₂; 200 μM each of dATP, dGTP, dCTP, dTTP (a mix of unlabeled and [3H]dTTP); 12.5 μg activated salmon sperm DNA, in a final volume of 50μl.

PCR Colored 5xReaction Buffer with 5x1.5mM Mg²⁺ (supplied with the GemTaq™ Hot Start DNA Polymerase)

The Colored 5xReaction Buffer for amplifications was specially formulated to provide direct loading of the PCR samples onto gels without the need to add a loading sample buffer. It contains a compound that increases sample density for electrophoresis and two dyes (Red and Yellow) that separate during electrophoresis to allow for easy monitoring. The Red and Yellow dyes migrate at the same rate as 800-1000bp DNA fragments and 20-30bp DNA fragments in a 1% agarose gel, respectively.

Note. If precipitation occurs during storage at freezing temperatures, it should be dissolved before usage.

Storage and Dilution Buffer

20 mM Tris-HCl (pH 7.5 at 25°C), 100 mM NaCl, 0.1 mM EDTA, 2 mM DTT, 50% Glycerol and 0.1% Tween-20.

Storage Conditions

GemTaq™ Hot Start DNA Polymerase can be stored for 6 months at -20°C.

Basic PCR Protocol

(For amplifications of DNA fragments shorter than 1000 bp)

1. Vortex gently GemTaq™ Hot Start DNA Polymerase to mix it and spin down content of the tube before adding it to the Reaction Mix. Thaw all reagent solutions completely, vortex thoroughly before use and keep all components on ice.
2. Combine the components of the Reaction Mix in the tubes for PCR (A). Recommended final concentrations of components in each sample for PCR amplifications are: 1xReaction Buffer (with 1.5mM Mg²⁺); 200μM of each dNTP (provided by the user); 0.2-0.4μM of each primer (provided by the user); template DNA (provided by the user); nuclease-free water (provided by the user) and 1unit of GemTaq™ Hot Start DNA Polymerase (B) in a 50μl reaction volume (C). Mix the content of each PCR sample gently.
3. If using a thermal cycler without a heated lid, overlay the reaction mix with 1-2 drops of Mineral Oil. Centrifuge the reaction mix in a microcentrifuge for 5 seconds.
4. **Guidelines for Thermal Cycling program.** (Researchers can use their own protocols for specific applications.)

1 cycle	Denaturation	94-95 °C	3 min
	Annealing and elongation	72 °C	2-4 min
20-40 cycles	Denaturation	94-95 °C	15-30 sec
	Annealing	56 °C (D)	15-30 sec
	Elongation	72 °C (E)	0.5-1 min
Post-PCR (to finish extension of all templates)	Elongation	72 °C	5 min
Store		4° C	until analysis of samples

Notes

(A) The use of reaction master mixes is strongly recommended to avoid pipetting errors due to addition of small volumes (GemTaq™ Hot Start DNA Polymerase). Combine the appropriate multiples of the components (water, 5xReaction Buffer, dNTPs, primers and GemTaq™ Hot Start DNA Polymerase), make aliquots and start reaction by adding the template.

(B) For most routine applications 1 unit of enzyme will be more than enough. PCR amplifications of low template concentrations or which produce products greater than 2 kb may require optimization of these conditions and higher amounts (1.5-2 units) of GemTaq™ Hot Start DNA Polymerase.

(C) Negative controls are recommended for each set of the experiments, when instead of template DNA, an equivalent volume of nuclease-free water is added to one of the samples.

(D) The annealing temperature for a specific amplification reaction will depend upon the sequences of the two primers and could vary from 50°C to 65°C based on the primer T_m.

(E) Extension of the primer by GemTaq™ Hot Start DNA Polymerase requires approximately 0.5-1 minute per 1000bp of the template to be amplified.

WARNINGS

We recommend the use of lab coats, gloves and eye protection when working with these reagents. MGQuest assumes no liability for any damage resulting from handling or from contact with the above products.

LICENSES/PATENTS

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