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GemTaq™ DNA Polymerase and 5xReaction Buffer (with 5x1.5 mM Mg^{2+})

(Catalog #EP012, #EP013, #EP014, #EP015)

FOR LABORATORY USE ONLY

Description

GemTaq™ DNA Polymerase 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™ DNA Polymerase is a thermostable enzyme with a molecular weight approximately 94 kDa. It is able to withstand repeated heating to 95°C without significant loss of activity. GemTaq™ DNA Polymerase has 5'→3' DNA polymerase activity and 5'→3' exonuclease activity, the same fidelity as *Taq* polymerase and can be substituted in protocols that currently use *Taq* polymerase. The enzyme can amplify fragments of up to 5 kb in length from genomic DNA and longer fragments from Lambda DNA. GemTaq™ DNA Polymerase is supplied at 5u/µl and comes with an optimized 5xReaction buffer. The purified enzyme has no detectable endonuclease or exonuclease activity.

Advantages

- Higher efficiency and sensitivity in a wide range of PCR assays when compared to a standard *Taq* DNA Polymerase.
- Suited for amplifications of long DNA fragments.
- Unbeatable value in terms of cost per unit and performance.

Applications

- Variety of PCR assays
- Primer extension
- DNA microarray analysis
- 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 $MgCl_2$; 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 5xReaction Buffer with 5x1.5mM Mg^{2+} (supplied with the GemTaq™ DNA Polymerase)

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™ DNA Polymerase can be stored for 12 months at -20°C.

Basic PCR Protocol
(For amplifications of DNA fragments shorter than 1000 bp)

1. Vortex gently GemTaq™ 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 Mg^{2+}); 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™ 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	2-3 min
	Annealing and elongation (Hot Start – user choice),	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™ DNA Polymerase). Combine the appropriate multiples of the components (water, 5xReaction Buffer, dNTPs, primers and GemTaq™ 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™ 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 Tm.
(E) Extension of the primer by GemTaq™ 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.

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A purchase of this product does not include a license to perform any patented application.