

Description: Polymerase Chain Reaction (PCR) is an effective tool to synthesize defined sequences of DNA using thermostable polymerases. PCR exploits the inherent stability of these enzymes at high temperatures to amplify about a million copies of desired DNA fragment using two specific oligonucleotide primers that anneal to opposite strands flanking the target DNA sequence to be amplified.

PCR is now widely used in cloning and sequencing DNA fragments, as a diagnostic tool and to generate labelled probes. To cater to these varied applications, Bangalore Genei has a range of PCR enzymes, buffers, kits and optimized protocols.

1. **Taq DNA Polymerase:** Taq DNA Polymerase, a thermostable enzyme used in most routine PCR amplifications for sizes ranging from 100 bp to 3 kb, is a highly pure enzyme that lacks 3'-5' exonuclease (proof reading) activity. The enzyme can also be used in applications like RAPD, RFLP and AFLP studies to detect variations within the genomes of species.
2. **GeNei™ HotStart Taq DNA Polymerase:** GeNei™ HotStart Taq DNA Polymerase, used for improved PCR specificity, is an optimized mixture of highly purified Taq DNA Polymerase with high affinity anti-Taq monoclonal antibody that inhibits polymerase activity at sub optimal temperatures by binding to Taq DNA Polymerase.
3. **PR Polymerase:** PR Polymerase, used in PCR reactions that require high fidelity synthesis, is a thermostable enzyme that exhibits strong ProofReading activity. The 3'-5' exonuclease mediated proofreading activity enables the enzyme to repair mis-incorporated nucleotides and reduce errors during polymerisation. This enzyme is ideal for high fidelity amplifications of short stretches of target DNA (< 1.5 kb).

An improved and more defined range of long PCR enzymes with higher fidelity is now made available to the scientists. For higher sensitivity and better yield, we have two sets of long PCR enzyme systems.

4. **XT-5 PCR System:** XT-5 PCR System, a unique enzyme blend that utilizes the powerful processivity of Taq DNA polymerase and the ProofReading activity of PR polymerase. This system is particularly useful for amplifications of long target DNA from genomic DNA templates (guaranteed upto 5kb).
5. **XT-20 PCR System:** XT-20 PCR System, a unique enzyme mix optimised for amplification of longer target DNA (guaranteed upto 20 kb) from genomic DNA templates.

Quality Control tests for Thermostable Polymerases

Unit Definition:

One unit is defined as the amount of enzyme which incorporates 10 nanomoles of total deoxyribo-nucleoside triphosphates into acid precipitable DNA in 30 minutes at 75° C under optimal assay conditions.

Assay for non-specific endonuclease:

1 µg of supercoiled DNA incubated with excess enzyme at 37°C and 72°C for 16 hours showed no relaxation of supercoiled DNA when visualised on a 1% agarose gel. (400 µM each of dATP, dCTP and dGTP were supplemented to reaction mix in case of PR polymerase and XT systems).

Assay for exonuclease activity:

1 µg of lambda DNA digest incubated with excess enzyme at 37°C and 72°C for 16 hours showed no alteration in banding pattern when visualised on a 1% agarose gel. (400 µM each of dATP, dCTP and dGTP were supplemented to reaction mix in case of PR polymerase and XT systems).

Performance test:

Performance tested as mentioned in product description of each enzyme.

Purification:

All thermostable polymerases are purified to near homogeneity using procedures developed in-house.

Thermostability:

Taq DNA polymerase and PR polymerase are highly thermostable enzymes. They retain >90% activity even after prolonged incubation (1 hour) at 94°C.

Stability:

All batches of thermostable polymerases are periodically tested for activity and are found to be stable for more than 12 months when stored at -20°C. Exposure to temperature greater than -20°C should be minimised.

Patents/Disclaimer:

Some products/processes mentioned in this catalogue may be covered by patents issued and applicable in certain countries for certain periods. Because purchase of our products does not include a licence to perform patented applications (in these countries) users have to ensure that they have the necessary licence for use.