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# E Taq DNA Polymerase

**Product Number: PC88**

## Shipping and Storage

-20°C

## Components

| Component | PC88 500U | PC88 2500U | PC88 10000U |
|---|---|---|---|
| E Taq DNA Polymerase, 5U/μl | 100μl | 5×100μl | 2×1ml |
| 10×PCR Buffer | 1.8 ml | 5×1.8ml | 8×5ml |

Note:The 10×PCR Buffer of this product contains 15mM $Mg^{2+}$.

## Description

E Taq DNA Polymerase is an optimized mixed enzyme of Taq and Pfu DNA Polymerase, with 5'→3' DNA polymerase activity, 5'→3' exonuclease activity, and 3'→5' exonase activity. Compared with Taq DNA Polymerase, E Taq DNA Polymerase has excellent performance of high amplification efficiency and low mismatch rate, and can efficiently amplify DNA fragments. Most of the PCR products amplified with this product have an "A" base at the 3'end, which can be directly used for T/A cloning. This product is suitable for routine PCR reactions and gene cloning with high fidelity requirements.

## Unit definition

Using activated salmon sperm DNA as template/primer, the amount of enzyme required to incorporate 10nmol of deoxynucleotides into acid insoluble substances within 30 minutes at 74°C was defined as 1 activity unit (U).

## Quality Control

After several column purifications, the purity of the SDS-PAGE test is more than 99%; no exogenous nuclease activity is detected; no host residual DNA is detected by PCR method; it can effectively amplify single-copy genes in the human genome; it can be stored at room temperature for one month , no significant activity changes.

## Protocol

The following example is a PCR reaction system and reaction conditions for amplifying a 1kb fragment using human genomic DNA as a template. In actual operation, corresponding improvement and optimization should be carried out according to the different template, primer structure and target fragment size.

1. PCR reaction system

| Reagent | 50μL reaction system | Final Concentration |
|---|---|---|
| 10×PCR Buffer | 5μl | 1× |
| dNTP Mix，10mM each | 1μl | 200μM each |
| Forward Primer，10μM | 2μl | 0.4μM |
| Reverse Primer，10μM | 2μl | 0.4μM |
| Template DNA | <0.5μg | <0.5μg/50μl |
| E Taq DNA Polymerase，5U/μl | 0.25-0.5μl | 1.25-2.5 U/50μl |
| $ddH_2O$ | up to 50μl | |

Note:Please use the final concentration of 0.1-1.0μM as the reference for the setting range of primer concentration. When the amplification efficiency is not high, the concentration of primers can be increased; when non-specific reactions occur, the concentration of primers can be decreased to optimize the reaction system.

2. PCR reaction condition

| Step | Temperature | Time | |
|---|---|---|---|
| Predenaturation | 94°C | 2min | |
| Denaturation | 94°C | 30s | 25-35cycles |
| Annealing | 55-65°C | 30s | |
| Extend | 72°C | 30s | |
| Final extension | 72°C | 2min | |

Note:1)In general experiments, the annealing temperature is 5°C lower than the melting temperature Tm of the amplification primer, and when ideal amplification efficiency cannot be achieved, the annealing temperature should be appropriately reduced; When a non-specific reaction occurs, increase the annealing temperature to optimize the reaction conditions.

2)The extension time should be set based on the size of the amplified fragment, and the amplification efficiency of this product E Taq DNA Polymerase is 2 kb/min.

3)The number of cycles can be set based on the downstream application of the amplification product. If the number of cycles is too small, the amplification amount is insufficient; If there are too many cycles, the probability of mismatch will increase, and the non-specific background will be severe. So, while ensuring product yield, the number of cycles should be minimized as much as possible.