# Product Information

## exTEN 2X PCR Master Mix, 1000 reactions

**C/No.** BIO-5186-1000
**Concentration** 0.08U/µl exTEN DNA Polymerase, 400µM dNTP mix, 3mM $MgCl_2$

**Packaging** 20 x 1.25ml
**Storage** -20°C
Avoid frequent thawing and freezing.

**Axil Scientific Pte Ltd**
41 Science Park Road
#01-22/23 The Gemini
Singapore Science Park II
Singapore 117610

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## Description

exTEN 2X PCR Master Mix is a unique premixed blend of Taq DNA Polymerase, a proofreading enzyme, dNTPs, MgCl2 and reaction buffer for amplification of DNA templates by PCR. With →the addition of a 3' 5' exonuclease (proofreading) ability, the amplification efficiency is enhanced through a lower error rate of misincorporated nucleotides compared to just Taq DNA Polymerase alone. exTEN 2X PCR Master Mix produces higher yields and amplifies longer fragments up to 10kb. Most the amplified DNA fragments have 3'A overhang, while a small percentage are blunt-ended. This premix formulation saves times and reduces contamination by reducing the number of pipetting steps for PCR setup. exTEN 2X PCR Master Mix consists of a density reagent and 2 tracking dyes which migrates at the same rate as a 4000bp and 50bp DNA fragment in a 1% agarose gel.



## Unit Definition

One unit of the enzyme catalyzes the incorporation of 10nmol of deoxyribonucleotides into a polynucleotide fraction in 30min at 74°C.

## Applications

• DNA sequencing • DNA labeling • PCR for cloning
• PCR amplification of DNA fragments up to 10kb

## exTEN 2X PCR Master Mix Composition

Contains 0.08U/µl exTEN DNA Polymerase, 400µM dNTP Mix, 3mM $MgCl_2$, reaction buffer and a PCR enhancer.



## Quality Control

Nuclease Assay
No detectable contaminating endonuclease or exonuclease activity.

Functional Assay
PCR Master Mix was tested for amplification of 3495bp of human genomic DNA.

**Certified by:**

**Noreeman Abdul Manan**
*Laboratory Officer*

## Protocol

The following protocol serves as a starting point and general guideline for any Polymerase Chain Reaction (PCR). Reaction conditions such as incubation time and annealing temperature may vary and need to be optimized. PCR reactions should be prepared in DNA-free environment, dedicated pipette and aerosol resistant tips are recommended. Always keep the control and template DNA isolated from other components.

**Recommendations of Template DNA in a 50 µl reaction volume**

| DNA Type | Amount of DNA |
|---|---|
| Human genomic DNA | 0.1 to 1 µg |
| Plasmid DNA | 0.5 to 5 ng |
| Phage DNA | 0.1 to 10 ng |
| *E.coli* genomic DNA | 10 to 100 ng |

1. Thaw exTEN 2X PCR Master Mix at room temperature. Keep the exTEN 2X PCR Master Mix on ice after thawing. Vortex briefly and spin down contents quickly.
2. Prepare the following reaction mix in a sterile, nuclease-free PCR tube on ice, overlay with 50µl of mineral or silicone oil.

| **For a 50µl reaction volume:** | | |
|---|---|---|
| **Components** | **Volume (µl)** | **Final Conc.** |
| exTEN 2X PCR Master Mix | 25 | 1X |
| Forward Primer (10µM) | 0.5 – 5 | 0.1 – 1µM |
| Reverse Primer (10µM) | 0.5 – 5 | 0.1 – 1µM |
| DNA template | 1 – 5 | See above |
| Nuclease-Free water | Top up to 50 | N.A |

3. Cap tubes and spin down contents briefly.
4. Place reactions in thermal cycler and incubate at 95°C to completely denature template DNA. Perform about 25 – 35 cycles of PCR amplification (repeat steps 2 to 4 for 25 - 35 cycles). Low amounts of starting template may require 40 cycles.

**Recommended PCR Cycling Condi on**

| No. | Step | Temperature | Time | |
|---|---|---|---|---|
| 1 | Ini al Denatura on | 95°C | 2 - 4 min | |
| 2 | Denatura on | 95°C | 30 seconds | 25 – 35 cycles |
| 3 | Annealing | 42 - 65°C | 30 seconds | |
| 4 | Extension | 68°C* / 72°C | 1 min/kb | |
| 5 | Final Extension | 68°C* / 72°C | 5 – 10 min | |
| 6 | Soak | 4°C | Several hours | |

*If thermal cycler has a refrigeration or "soak" cycle, cycling reaction can be programmed to end by holding the tubes at 4°C for several hours.

## General guidelines for PCR amplifications

Initial denaturation
To ensure efficient utilization of the template during first amplification cycle, it is essential that the template is denatured completely. If GC content of the template is ≤ 50%, an initial 1 – 5 min denaturation at 95°C would be sufficient.

Denaturation
DNA denaturation time of 0.5min/cycle at 95°C is usually sufficient. For GC-rich templates, denaturation could be prolonged to 3 – 4 min.

Annealing
Annealing temperature should be 5°C lower than melting temperature ($T_m$ - 5°C) of primers. 0.5min/cycle is usually sufficient. If non-specific PCR products are observed, the temperature should be optimized stepwise in 1-2°C increments.

Extension
Optimal temperature for extension of *Taq* DNA Polymerase is between 70-75°C. Recommended extension step is 1min/kb at 72°C for PCR products. *For >5kb amplification, it is recommended to use 68°C as the extension temperature.

Number of cycles
If less than 10 copies of template are present, about 40 cycles are required. For higher amount, 25-35 cycles are sufficient.

Final extension
After the last cycle, incubate PCR mixture at 72°C for an additional 5 – 15 min to allow any possible incomplete amplification to take place.