exTEN II PCR Master Mix (2X)

Cat. No.: BIO-5188-20
BIO-5188-200
BIO-5188-1000

Concentration: 0.06U/µl exTEN II DNA polymerase, 400uM dNTP mix, 3mM $MgCl_2$

Storage: -20°C.
Avoid frequent thawing and freezing.

Quality Control

Nuclease Assay: No detectable contaminating endonuclease or exonuclease activity.

Functional Assay: exTEN II PCR Master Mix (2X) was tested for amplification of 12000bp of human genomic DNA.

1.0 DESCRIPTION

exTEN II PCR Master Mix (2X) is a unique premixed blend of Taq II 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 II DNA Polymerase alone. exTEN II PCR Master Mix (2X) produces higher yields and amplifies longer fragments up to 12kb. Most of the amplified DNA fragments have 3'A overhang, while a small percentage are blunt- ended. This premix formulation saves time and reduces contamination by reducing the number of pipetting steps for PCR setup. exTEN II PCR Master Mix (2X) consists of a density reagent and 2 tracking dyes which migrate 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 an acid-insoluble material in 30mins at 74°C by using activated salmon sperm DNA as a template / primer.

Applications

- DNA sequencing
- DNA labeling
- PCR for cloning
- PCR amplification of DNA fragments up to 12kb
- Routine PCR

exTEN II PCR Master Mix (2X) Composition
Contains 0.06U/µl exTEN II DNA Polymerase, 400µM dNTP Mix, 3mM $MgCl_2$, reaction buffer and a PCR enhancer.

Remarks

- Half-life of this enzyme is >40mins @ 95°C.
- Taq II DNA Polymerase accepts modified nucleotides (e.g. biotin-, digoxigenin-, fluorescent-labeled nucleotides) as substrates for the DNA synthesis.

2.0 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 a 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 II PCR Master Mix (2X) at room temperature. Keep the tube on ice after thawing.
2. Vortex briefly and spin down contents quickly. Prepare the following reaction mix in a sterile, nuclease-free PCR tube on ice.

Table 1. For a 50µl reaction volume:

Components	Volume (µl)	Final Concentration
exTEN II PCR Master Mix (2X)	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 25 - 35 cycles of PCR amplification by repeating steps 2 to 4 in the PCR reaction. Low amounts of starting template may require 40 cycles.

Table 2. PCR Amplification as follows:

Step	Temperature	Time
Initial Denaturation	95°C	2 – 4 min
Denaturation	95°C	30 seconds
Annealing	42- 65°C	30 seconds
Extension	*68°C / 75°C	**0.5 – 1 min/kb
Final Extension	*68°C / 75°C	5 – 15 min
Soak	4°C	∞

*For >5kb amplification, it is recommended to use 68°C as the extension temperature.
**For >3kb amplification, it is recommended to use 1kb/min as extension rate

3.0 General guidelines for PCR amplifications

Initial denaturation

To ensure efficient utilisation of the template during the first amplification cycle, it is essential that the template is denatured completely. If GC content of the template is ≤ 50%, an initial 1-5 mins 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 3-4mins.

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 optimised stepwise in 1-2°C increments.

Extension

Optimal temperature for extension of exTEN II DNA Polymerase is between 70-75°C. Recommended extension step is 30sec/kb at 72°C for PCR products.

Number of cycles

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

Final extension

After the last cycle, incubate PCR mixture at 72°C for an additional 5-15 mins to allow any incomplete amplification to take place.