Differentiating Research

Product Details

Extremo™ MasterMix (2X)

For Routine PCR and 2nd Strand Synthesis from cDNA

Product Overview

This is 2X concentrated solution for PCR reaction which contains dNTPs, **Taq DNA Polymerase**, Mg+2 and other critical reaction components. It doesn't contain primers and DNA template. Extremo Mastermix is designed for routine PCR and 2nd Strand Synthesis from cDNA. You can expect the amplification upto 8KB

Catalog Details

R2020	100 Reactions. With Gel Loading Dye	R2020A	100 Reactions. Without Gel Loading Dye
R2021	1000 Reactions. With Gel Loading Dye	R2021 A	1000 Reactions. Without Gel Loading Dye

Storage

- 20 ℃

PCR Protocol

The samples must be prepared in sterile DNase Free micro-centrifuge tubes, with the following composition placed on ice

Components	Example for 50µL reaction	Final Concentration
Template DNA/cDNA	2 µL	5ng - 100 ng
Forward Primer (10µM)	1 µL	0.1 - 1µM
Reverse Primer (10µM)	1 µL	0.1 - 1µM
Extremo MasterMix (2X)	25 µL	1X
Nuclease Free Water	Upto 50 µL	

Critical Note:

- Total volume of cDNA templates should not be more than 10% v/v of total reaction volume.
- cDNA template can also be diluted 10 times if copy numbers are very high

After the preparation of PCR mix samples as above, they are transferred to thermal cycler. The thermal cycling process begins with initial denaturation step. The denaturation process is to ensure complete denaturation of the target DNA at 95℃. The standard steps for thermal cycler are tabulated below with optimum temperature, time, and number of cycles. Generally, 25 – 45 cycles yield sufficient product.

Step	Temperature	Time	Cycle
Initial denaturation	95 ± 1 ℃	2 - 5 minutes	1
Denaturation	95 ± 1 ℃	0.5 - 1 minutes	25 - 35
Annealing *	45 - 65 ℃	0.5 - 1 minutes	
Extension	68 - 72 ℃	1 minute/kb	
Final Extension	68 - 72 ℃	5 - 15 minutes	1
Hold, if required.	2- 8 ℃	variable	1

If your mastermix contains green dye, then load it directly on the gel. The green dye will separate into blue (~4kb) and yellow (~25bp) in 1% agarose gel.

Extremo™ MasterMix (2X)

For Routine PCR and 2nd Strand Synthesis from cDNA

Critical Note

cDNA quality depends on the initial RNA template used. Few desired genes might have very low or very high transcripts based on the cell's growth conditions. Users can empirically choose 25 to 45 cycles in the PCR step to obtain desired amplification. For very low copy transcripts use 45 cycles and for a high copy transcript you can use 25 or 30 cycles would suffice.

Quality Control Assays

1. **Purity:** SDS Page analysis with Coomassie Blue Staining resulted in ≥ 95% purity.
2. **Performance testing 1:** In a 25µL reaction, 12.5µL of mastermix was used to amplify 1ng of DNA template (lambda) of various sizes (300bp, 1kb, 3kb, 5kb) with appropriate primers. PCR was run with 30 cycles resulted in a single band, confirmed by 1% agarose gel electrophoresis with EtBr.
3. **Performance testing 1:** In another test, 2µL of cDNA template was used for amplification after Reverse Transcription using PhiScript cDNA Synthesis Kit (R6201). Due to low copy number, 45 cycles was used. Amplicon size was 656bp and 3kb. Both were amplified and clear bands were visible on 1% agarose gel.
4. **Nuclease tests:** No contamination of endo or exonucleases were detected.

Any Technical Help ?

Please write to us at info@dxbidt.com . Response can be expected within 24Hrs. Our technical team shall be happy to assist you all the time.

Order Related Queries

Email : info@dxbidt.com | Ph: +91-7349708807

Website: https://dxbidt.com

Address: #87, Dasanapura, Lakshmipura Post, Bangalore - 560073