2X Hot start mix

Cat No	Pack size	conc
HTM0500	3X5 ml	2x
HTM2500	5x5ml	2x

Description :Hot start Taq DNA Polymerase for qPCR is designed for Real-Time PCR and Hot-start PCR. A special inhibition the reaction at room temperature until after the first denaturation step. This prevents primer-dimers and other artefacts.

2X Hot Start mix is optimized mixture contain of Hot Start Taq enzyme, reaction buffer, dNTP And enhancer as 2-fold concentration. 2x Hot Start mix is designed to allow the user for quick ,easy preparation of reaction mixture. The 2x Hot Start mix can be amplification PCR products up to 5 kb and the products can be directly cloning into T-vector.

storage conditions: long time at -20˚C
short time at 4 ˚C

Template

2 x Hot start mix is suitable for amplifying targets up to 3 kb from the following templates:

Genomic DNA: 10–200 ng
Plasmid DNA : 1–5 ng
cDNA : ~100 ng starting total RNA

Primers

Use 0.3 μM per primer as a general starting point. For larger amounts of template (e.g., 200 ng genomic DNA), increasing the concentration up to 0.5 μM per primer may improve yield.

Annealing Temperature

The annealing temperature is slightly higher than with typical PCR. The optimal annealing temperature should be ~2˚C lower than the Tm of the primers used. A range of 58–68˚C is recommended.

Extension Time: As little as 30 seconds per kb is suitable for most targets. Use up to 60 seconds per kb for maximum yield.

PCR Protocol:

1.Thaw the 2x Hot start mix at room temperature. Vortex the 2x Hot start mix and then spin it briefly in a micro centrifuge to collect the material in the bottom of the tube.
2. Prepare one of the following reaction mixes on ice:

Component	Volume
2x Hot start mix	12.5 ul
Primer1 (20 pmol)	1-2 ul
Primer2 (20 pmol)	1-2 ul
template	1-10 ul
ddH_2O	Up to 25 ul
Total	25 ul

3. If necessary you can scale up your volume

1. Program the thermal cycler as follows:

Step	Temperature	Time	Cycle
Initial denaturation	94-95°C	10 mins	1
Denaturation	94-95°C	0.2-2mins	20-35
Annealing	50-68	0.2-2mins	
Extension	72	1min/1kb	
Final extension	72	1-10mins	1

Step

After cycling, maintain the reaction at 4˚C. Samples can be stored at –20˚C until use.

Analyze products using standard agarose gel electrophoresis.

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