2X Hot Start Master Mix for PCR/qPCR

Product Insert

FOR RESEARCH USE ONLY

Catalog No.: AG314-1 (1 x 1 ml)
AG314-5 (5 x 1 ml)

Introduction:
Denovo Biotechnology's 2X Hot Start PCR Master Mix is a ready-to-use solution for PCR and quantitative PCR. It contains Taq DNA polymerase, an aptamer that inhibits Taq polymerase activity at low temperatures, dNTPs, and Taq polymerase buffer.

Components of 2X PCR Master Mix:

1. Taq polymerase 50 U/ml
2. Oligonucleotide aptamer inhibitor 20 nM
3. dNTPs (600 uM each)
4. Taq buffer (20 mM Tris pH 8.6, 100 mM KCl, 6mM MgCl2)

Materials Required but not supplied:

1. Primers specific for the gene of interest. For PCR amplification it should contain a forward and a reverse primer. For quantitative PCR you may use a primer/probe mix containing a forward primer, a reverse primer, and an internal primer labeled with a fluorescent reporter dye and a quencher dye. These probes are commercially available and usually sold at a 20X concentration.
2. Nuclease-free H2O
3. DNA samples (genomic DNA, viral DNA, plasmid or cDNA) isolated and purified by the procedure of your choice, dissolved in nuclease-free H2O
4. For quantitative PCR, a real-time PCR detection system capable of detecting emission from the fluorescent dye.

Procedure:
Combine the reaction components on ice or at room temperature. Reaction volumes can be 10 ul to 50 ul. Add sufficient amount of 2X Real Time PCR Master Mix to achieve a final concentration of 1X. A sample 25 ul reaction is shown below:

- For PCR, forward and reverse primers added to achieve a final concentration of 0.2 – 1 uM each. For quantitative PCR, add 1.25 ul of a 20x probe mix specific for the gene of interest. These can be purchased from several sources such that the final concentrations of forward and reverse primers will be 0.2 – 1uM each, and the dual-labeled probe will be at a final concentration of 50-250 nM.
- 12.5 ul 2X Real Time PCR Master Mix
- Template DNA (10 ng -1 ug genomic DNA, or 0.5 pg – 5 ng plasmid/viral DNA, or 1 – 100 ng cDNA)
- Nuclease-free dH2O to final volume of 25 ul.

To minimize pipetting errors when performing quantitative PCR, it is recommended that the first three reagents are combined in a sufficient quantity for all of the standards and samples to be assayed. Then distribute the appropriate volume into each reaction tube, and add the template DNA.
This final mixture can then be added to the wells of the plate to be used in the real-time PCR detection device. We recommend performing multiple repeats of each sample, if possible.
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The optimal thermocycling conditions can be determined empirically, but the suggested parameters for routine PCR are as follows:
1. Initial Denaturation Step: 95°C 2 min.
2. 40 cycles of:
    a. Denature: 95°C 20 s
    b. Anneal/Extension: 60°C 1 min/kb
3. Final Extension 68°C 5 min

The following conditions are recommended for all Denovo Biotechnology qPCR assays:

1. Initial Denaturation Step: 95°C 2 min.
2. 40 cycles of:
    a. Denature: 95°C 20 s
    b. Anneal/Extension: 60°C 45 s.