DNAbiotech

Biotechnology is our expertise

2X Blue Load PCR Master Mix

Catalog no.: DB9705

1 ml and 5 × ml

Related product: 2X PFU master mix

Intended for Research Use Only

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Diba NoAvaran Azma Company

Customer and technical support

If you have any question, do not hesitate to ask! DNAbiotech would be highly appreciated for any comment(s).

Contact us at

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Quality Control

In accordance with DNAbiotech Co. Management System, each part of the product tested against predetermined specifications to ensure consistent product quality**.**

General description

"DNAbiotech Blue Load Taq Master Mix" contains an inherent blue dye and allows the direct loading of the PCR reaction product onto the agarose gel. It contains all reagents required for PCR (except template and primer) in a premixed 2x concentrated ready-to-use solution. The Master Mix is recommended for use in routine PCR reactions. It is optimized for high specificity and guarantees minimal by-product formation. The mix is particularly suitable for plate based PCR and automated pipetting where a detergent free buffer system is required. It catalyzes the polymerization of nucleotides into duplex DNA in 5'→3' direction in the presence of magnesium. It also possesses a 5'→3' polymerization dependent exonuclease replacement activity but lacks a 3'→5' exonuclease activity. This product contains a reaction buffer, 2mM Mgcl2, dNTPs and a thermostable DNA polymerase and protein stabilizer. **"DNAbiotech Blue Load Taq Master Mix"** has been optimized for use in routine PCR reactions for amplifying DNA template in the range of 0.1–3kb.

Product Information

Cat #: DB9705

Volume: 1 ml and 1×5 ml

Storage condition: at -20°C for 24 months

Applicatins**:**

- Routine PCR
- Amplification of DNA for Sanger sequencing
- Primer Extension
- Microarray Analysis
- High-Throughput PCR
- Hot-start PCR
- Diagnostic PCR

General Guidelines for Amplification by PCR

The following guidelines apply to target sequences between 200 and 2,000bp and are optimal for typical thermal cyclers.

A. Denaturation

• Generally, a 2-minute initial denaturation step at 95°C is sufficient.

• Subsequent denaturation steps will be between 30 seconds and 1 minute.

B. Annealing

• Optimize the annealing conditions by performing the reaction starting approximately 5°C below the calculated melting temperature of the primers and increasing the temperature in increments of 1°C to the annealing temperature.

•The annealing step is typically 30 seconds to 1 minute.

C. Extension

•The extension reaction is typically performed at the optimal temperature for Taq DNA polymerase, which is 72–74°C.

•Allow approximately 1 minute for every 1kb of DNA to be amplified.

•A final extension of 5 minutes at 72–74°C is recommended.

D. Refrigeration

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

•This cycle can minimize any polymerase activity that might occur at higher temperatures, although this is not usually a problem.

E. Cycle Number

• Generally, 25–30 cycles result in optimal amplification of desired products.

•Occasionally, up to 40 cycles may be performed, especially for detection of low-copy targets.

Basic procedure:

Note:This optimization is basic and the researcher can change it according to desired volume.

1. Thaw DNAbiotech 2x PCR master mix before use. To avoid repeated freeze-thaw it is advised to aliquot the content of the vial according to your desired volume.

2. Prepare a master mix as following for a 20 µl reaction volume:

Ingredients	Volume	Final concentration
2X *Taq* Premix	10 µL	1X
Reverse Primer (10 pmol/ µL)	1 ul	0.5pmoles/µL
Forward Primer (10 pmol/ µL)	1 ul	0.5pmoles/µL
Template oligo.	Variable	10 ngr- 1 ugr
Sterilized Distilled water	Variable	-
Total Vol.	20 ul	

3. Mix and dispenseappropriate volumes into PCR reaction tubes.

4. Spin the reactions tubes in a microfugefor 5 seconds.

5. Perform PCR using your own standard method or use the following table:

No. ofcycles	Time	Temperature°C
1	5 minutes	94
25 to 35	30 seconds	94
	30 seconds	About 55 (should be set up)
	About 50-60 seconds	72
1	10 minutes	72

6. Separate the PCR products by agarose gel electrophoresis and visualize with EtBr or by any other dyes.

Note: For longer DNA target, extension time should increase to approximately 1 min/per Kb DNA.