MQ HotStart Ready Mix

Cat. No. HM-M-002-1250
Size: 1.25ml
Store at -20°C

MOLEQULE-ON®

Kit Components	Quantity
MQ HotStart Ready Mix	1.25 ml

Description

The MQ HotStart Ready Mix is a combination of *Taq* DNA polymerase and an engineered archaeal (B-family) DNA polymerase. Both enzymes possess 5' – 3' polymerase activity, but only *Taq* possesses 5' – 3' exonuclease activity, and only the B-family DNA polymerase possesses 3' – 5' exonuclease activity. This two-enzyme system is designed to support robust, long-range, and sensitive PCR.
In the MQ HotStart formulation, the enzyme mixture is combined with an exclusive antibody that inactivates the enzyme until the first denaturation step. This eliminates non-specific amplification products resulting from non-specific primer binding during reaction setup and initiation, and increases overall reaction efficiency.
MQ HotStart Ready Mix with 2X dye is a ready to-use mixture containing all components for a standard to long-range PCR, except primers and template. The 2X Ready Mix contains MQ DNA Polymerase Mix (0.2 U per µl reaction), MQ Buffer (1X), dNTPs (0.4 mM of each dNTP at 1X), $MgCl_2$ (4 mM at 1X) and stabilizers.
MQ 2X Ready Mix also contains two inert tracking dyes to enable direct loading of PCR products onto agarose gels for analysis by electrophoresis, without the need to add a DNA loading buffer.

Applications

The MQ HotStart Ready Mix is ideally suited for:
- Routine PCR
- PCR using complex templates
- (Single Nucleotide Polymorphism) SNP Analysis
- Any standard PCR application for which a hot start formulation of a high-quality thermostable DNA polymerase is required.

Procedure

Step 1: Prepare the PCR master mix

Ensure that all reagents are properly thawed and mixed.
1. Prepare a PCR master mix containing the appropriate volume of all reaction components common to all or a subset of the reactions to be performed.
2. Calculate the required volumes of each component based on the following table:

Components	25 µl reaction	Final Conc.
PCR-grade water	Up to 25 µl	N/A
HotStart ReadyMix with dye	12.5 µl	1X
Forward Primer (10 µM)	1.25 µl	0.5 µM
Reverse Primer (10 µM)	1.25 µl	0.5 µM
Template DNA*	As required	As required

* ≤100 ng for genomic DNA; ≤1 ng for less complex DNA (e.g. plasmid, lambda).

Step 2: Set up individual reactions

1. Transfer the appropriate volume of PCR master mix, template and primer to individual PCR tubes/wells or a PCR plate.
2. Cap or seal individual reactions, mix and centrifuge briefly.

Step 3: Run the PCR

Perform PCR with the following cycling protocol:

<table>
<tr><th>Step</th><th>Temperature</th><th>Duration</th><th>Cycle</th></tr>
<tr><td>Initial denaturation</td><td>95 ºC</td><td>3 min[1]</td><td>1</td></tr>
<tr><td>Denaturation</td><td>95 ºC</td><td>15 sec</td><td rowspan="3">20-40 [4]</td></tr>
<tr><td>Annealing[2]</td><td>Tm - 5 ºC</td><td>15-30 sec</td></tr>
<tr><td>Extension[3]</td><td>72 ºC</td><td>1 min/kb</td></tr>
<tr><td>Final Extension[5]</td><td>72 ºC</td><td>1 min/kb</td><td>1</td></tr>
<tr><td>Store</td><td>4-10 ºC</td><td>HOLD</td><td>1</td></tr>
</table>

1 Initial denaturation for 3 min at 95 °C is recommended for most assays. For GC-rich targets (>65% GC), 5 min at 95 °C may be used.
2 An annealing temperature 5 °C lower than the calculated melting temperature (Tm) of the primer set is recommended as a first approach. If low yields and/or nonspecific amplification is obtained, an annealing temperature gradient PCR is recommended to determine the optimal annealing temperature of the primer pair.
3 An extension temperature of 68 °C is recommended for long-range (5 kb to 10 kb) PCR products.
4 35 cycles are sufficient for most assays. A higher number of cycles may be necessary for assays requiring higher sensitivity, while lower cycle numbers can be used if the template copy number is high.
5 Final extension should be included if PCR products are to be cloned into TA cloning vectors.