FISHER MOLECULAR BIOLOGY

HOT START PCR 2X MASTER MIX (GC_Rich-buffer) FS-T-1141-1

Description

Hot Star -PCR 2X Master Mix is ready-to-use Hot-start PCR pre-mixes are the innovation for convenience of your routine PCR. The Hot Start -PCR 2X Master Mix is an optimized, ready-to-use PCR mixture of Hot Start-Taq DNA Polymerase, PCR buffer, $MgCl_2$,dNTP's, except DNA template and primers. The optimized buffer formula maximizes the effect of the enzyme, achieving low mismatch rate and high amplification efficiency for complextemplates. The original MasterMix formula makes the entire reaction system very stable and repeatable. This product already contains dyes, and the electrophoresis operation can be performed directly after the PCR procedure is completed. The amplified PCR product has an "A" base attached to the 3' end, so it can be used directly for T/A cloning. It is mainly suitable for the amplification of DNA templateswith high GC content

Kit Contents

Descripion	FS-T-1141-1	FS-T-1141-5
Hot Start -PCR 2X Master Mix with GC-Rich Buffer	5 ml	25 ml
ddH2O	5x1 ml	125x 1ml

1 ml=40 reactions

Applications

- High through-put PCR
- Hot-start PCR
- Routine diagnostic PCR requiring high reproducibility
- DNA sequencing template preparation
  GC-Rich DNA fragment sequencing

MgCl2 concentration: This product contains 3 mM MgCl2, which is suitable for most PCR reactions.

Storage Conditions

Store all components at -20ºC in a non-frost-free freezer.

Note

Do not contaminate the Hot Start -PCR 2X Master Mix with primers and template DNA used in individual reactions. Thaw and mix all compo-nents thoroughly, spin down shortly and chill on ice.

Quality Control

No endonuclease activity, nicking activity, exonuclease activity, or priming activity has been detected.

Recommended Protocol

This standard protocol applies to a single reaction where only template, primers, and water need to be added to the Hot Start -PCR 2X Master Mix. For multiple reactions, scale-up volume of reaction components proportionally. All reagents should be thawed on ice, gently mixed and briefly centrifuged before use.

1. Thaw reagents at room temperature. Mix thoroughly and then place on ice immediately after thawing.
2. Assemble reaction tubes on ice whenever possible to avoid premature, nonspecific polymerase activity.
3. The following table shows recommended component volumes:

Reaction Conditions

Component	25 ul reaction	50 µl reaction
Hot Start PCR 2X Master Mix With GC_Rich Buffer	12,5 µl	25µl
10µM Forward Primer	1 µl	2 µl
10µM Reverse Primer	1 µl	2 µl
Template DNA	≥ 1 µl	≥ 1 µl
Water, RNase-Free	Up to 25 µl	up to 50 µl

NOTE: Recommended amount of template per PCR reaction:

- **genomic DNA: 50-200 ng**
- **plasmid DNA: 0.1-10 ng**

4. Ensure reactions are mixed thoroughly by pipetting or gentle vortexing followed by a brief spin in a microcentrifuge.
5. Optional-Overlay reactions with one-half volume PCR-grade mineral oil when not using heated lid on thermal cycler.
6. Transfer tubes on ice into a thermal cycler pre-warmed. The following table shows recommended cycling conditions:

PCR Conditions

Step	Temp (˚C)	Time	Cycle
Pre-denaturation	94	5 min.	1
Denature	94	30 sec.	35
Anneal	50 ~60	30 sec.	
Extension	72	30 sec./kb	
Final Extension	72	10 min.	1

NOTE: Cycling conditions may need to be optimized, depending on different primer and template combinations. For example, raise the annealing temperature to prevent non-specific primer binding, increase extension time to generate longer PCR products.

7. **Annealing temperature:** Please refer to the theoretical Tm value of the primer, and the annealing temperature can be set lower than the theoretical value of the primer by 2-5°C.

8. **Extension time:**
   Molecular identification is recommended at 30 sec/kb. Gene cloning is recommended at 60 sec/kb to ensure the highest amount of product.

9. After cycling, maintain the reactions at 4°C or store at -20°C until ready for analysis.

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