FISHER MOLECULAR BIOLOGY

GOTAQ HOT START GREEN 2X MASTER MIX G2 FS-T-5141

Cat No.	Size
FS-T-5141	500 reactions 1000 reactions

Description

GOTAQ Hot Start Green Master Mix G2 is **2X Ready-to-Use** Hot-start PCR pre-mixes are the innovation for convenience of your routine PCR. The GOTAQ Hot Start Green 2X Master Mix G2 is an optimized, **Ready-to-Use** PCR mixture of **GOTAQ Hot Start Green** , **PCR buffer**, **$MgCl_2$** and **dNTP's**, except DNA template and primers.

The mixture is suitable for amplification of most of the DNA templates and highly processive 5'→3' DNA polymerase that lacks 3'→5' exonuclease activity and lacks a 3'→5' proofreading function. PCR reactions contains two dyes (blue and yellow) can be directly loaded onto an agarose gel without the additional need of loading buffer and dyes.

Contents	FS-T-5141
GOTAQ HS -PCR Green 2XMaster Mix G2	1 ml/100 reactions

Applications

GOTAQ Hot Start Green 2X Master Mix G2 is suitable and tested for amplification of genomic targets ranging from 100 bp to 4 kb and of episomal targets (lambda phage; plasmids) up to 10 kb under various reaction conditions.

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

Storage Conditions

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

Note

Do not contaminate the GOTAQ Hot Start Green 2X Master Mix G2 with primers and template DNA used in individual reactions. Thaw and mix all components thoroughly, spin down shortly and chill on ice.

Quality Control

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

Equivalent to GoTaq G2 Hot Start Polymerase

Recommended Protocol

This standard protocol applies to a single reaction where only template, primers, and water need to be added to the GOTAQ Hot Start Green 2X Master Mix G2. 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	20 µl reaction	Final Conc.
GOTAQ HS Green 2XMaster Mix G2	10 µl	1X
10µM Forward Primer	0.2 ~ 2.0 µl	0.1~1.0 µM
10µM Reverse Primer	0.2 ~ 2.0 µl	0.1~1.0 µM
Template DNA	1 ~ 5 µl	< 250 ng
Water, RNase-Free	up to 20 µl	

NOTE: In general, use greater than 0.5 µM primers for sensitivity and less than 0.5 µM for specificity.

NOTE: Recommended amount of template per PCR reaction:

- < 50 ng plasmid or
- < 500~1000 ng genomic DNA or
- 2 µl of a 100µl single plaque eluate or
- one single bacterial colony

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
Initial Denaturation	95	5 min.	1
Denature	95	10 ~ 60 sec.	25 ~ 40
Anneal	50 ~ 65	10 ~ 60 sec.	
Extend	72	60 sec./kb	
Final Extension	72	5 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. After cycling, maintain the reactions at 4°C or store at -20°C until ready for analysis.

For Research Use Only

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