2X PCR SensTaq HotStart MasterMix

Store at -20°C

Cat. No.	Description	Quantity
G939	2X PCR SensTaq HotStart MasterMix	5 ml
G939-dye	2X PCR SensTaq HotStart MasterMix with dye	5 ml

Product Description

crb's 2X PCR SensTaq HotStart MasterMix is a ready-to-use mixture containing **crb**'s SensTaq HotStart DNA Polymerase, dNTPs, and reaction buffer with proprietary additives in a 2X concentration. It contains all the necessary reagents for an efficient and sensitive amplification of DNA. The 2X PCR SensTaq HotStart MasterMix with dye (Cat. No. G939-dye) contains a green dye blend which resolves during gel electrophoresis into a turquoise band at ~4000 bp and a yellow band at the ~50 bp region. The resolving bands of dye allow easy visualization of the real time progress of your gel electrophoresis, and the yellow band indicates the migrating front in the gel.

SensTaq HotStart DNA Polymerase is a novel antibody-based hotstart DNA polymerase containing a dsDNA binding subunit. The hotstart capability of the enzyme gives its high specificity while the subunit boosts the enzyme's sensitivity and processivity. SensTaq HotStart DNA Polymerase has 5' to 3' exonuclease activity but lacks 3' to 5' proofreading activity, and it is able to amplify products upto 1.5 kb in length containing a single base (A) overhang on the 3' ends.

Features and Benefits

- Saves preparation time by combining SensTaq HotStart DNA Polymerase, dNTPs and reaction buffer into a ready-to-use mixture.
- Reduces the risk of contamination by decreasing the number of pipetting steps.
- Provides consistent reaction performance and results.

Shipping and Storage

Upon arrival, 2X PCR SensTaq HotStart MasterMix should be stored at -20°C. Avoid repeated freeze-thaw cycles of all components to retain maximum performance. All components are stable for 1 year from the date of shipping if stored and handled properly.

Protocol

The following basic protocol serves as a general guideline and starting point for any PCR amplification. Optimal reaction conditions (incubation times, temperatures, concentration of primers and template DNA) may vary and need to be optimized for each specific PCR.

All PCR experiments should be assembled in a nuclease-free environment. In addition, DNA sample preparation, reaction set-up and subsequent reaction(s) should be performed in separate areas to avoid cross contamination.

A negative control reaction (omitting template DNA) should always be performed in tandem with sample PCR to confirm the absence of DNA contamination.

1. Add the following components to a sterile 0.2 ml PCR tube sitting on ice.

Components	Volume	Final Concentration
Template DNA	~100 ng	~2 ng/µl
Forward primer (10 µM)	1 - 2.5 µl	200 - 500 nM
Reverse primer (10 µM)	1 - 2.5 µl	200 - 500 nM
2X PCR SensTaq HotStart MasterMix/ with dye	25 µl	1X
Nuclease-free H_2O	up to 50 µl	-

* Optimal Mg_{2+} concentration is specific to each DNA template-primer set and can only be determined experimentally.

- *We recommend preparing a mastermix for multiple reactions to minimize reagent loss and enable accurate pipetting.*

2. Mix contents of tube and centrifuge briefly.

3. Incubate tube in a thermal cycler at 94°C for 3 mins to completely denature the tem-plate.

4. Perform 30 - 35 cycles of PCR amplification as follows:

   Denature: 94°C for 30 sec

   Anneal: 45 - 72°C for 30 sec

   Extend: 72°C for 30-60 sec/1 kb template*

   * Optimal extension time can be seqeunce specific and can be determined experimentally. The speed of SensTaq HotStart DNA Polymerase is about 2kb/min and longer extension time up to 1kb/min can be used to further increase sensitivity.

5. Incubate for an additional 5 mins at 72°C and maintain the reaction at 4°C. The samples can be stored at -20°C until use.

6. Analyze the amplification products by agarose gel electrophoresis and visualize by ethidium bromide or SafeView™ (Cat No. G108) staining. Use appropriate molecular weight standards.