PCR Sure™ Kit

Cat. No. G065

Store at -20°C.

Description

It has been well established that many variables can affect a PCR including template structures, inhibitors, primer design, annealing temperature, and magnesium concentration. The PCR Sure™ Kit is developed to simplify the PCR optimization process. The system consists of multiple thermostable DNA polymerases pre-mixed with 8 optimized buffers in a 2X MasterMix format, saving a great amount of time in PCR set-up. To find the optimal conditions for your difficult PCR, all you need to do is mix templates, primers, and H_2O with the PCR Sure™ 2X MasterMixes.

Product Component	Quantity	Part No.
PCR Sure™ 2X MasterMix 1	10 rxn (125µl)	P065-1
PCR Sure™ 2X MasterMix 2	10 rxn (125µl)	P065-2
PCR Sure™ 2X MasterMix 3	10 rxn (125µl)	P065-3
PCR Sure™ 2X MasterMix 4	10 rxn (125µl)	P065-4
PCR Sure™ 2X MasterMix 5	10 rxn (125µl)	P065-5
PCR Sure™ 2X MasterMix 6	10 rxn (125µl)	P065-6
PCR Sure™ 2X MasterMix 7	10 rxn (125µl)	P065-7
PCR Sure™ 2X MasterMix 8	10 rxn (125µl)	P065-8

Additional Materials Required

- Template DNA
- Primers
- Nuclease-free H_2O

Protocol

1. Thoroughly thaw and mix individual components before use, and assemble reaction on ice. Prepare the Primer-Template Mix for 8 reactions as follows:

Component	Volume (x1)	Volume (x10)[1]
Forward Primer (10 µM)	0.5 µl	5 µl
Reverse Primer (10 µM)	0.5 µl	5 µl
Template DNA	1 µl (50 -100 ng/µl)	10 µl (50 -100 ng/µl)
Nuclease-free H_2O	10.5 µl	105 µl

[1]*extra volume to account for pipetting error*

2. Aliquot 12.5 µl of the Primer-Template Mix to each tube in a PCR strip, and then add 12.5 µl of each PCR Sure™ 2X MasterMix 1 – 8.
3. Gently mix the reaction components and briefly centrifuge. Transfer the strip to a thermalcycler and use thermocycling conditions for Touchdown PCR as follows:

Step	Temperature	Time	Cycles
Initial Denaturation	95°C	5 min	1
Touchdown 1	95°C Annealing Temperature[2] + 10°C 72°C	30 sec 30 sec 1 min/kb	5
Touchdown 2	95°C Annealing Temperature[2] + 5°C 72°C	30 sec 30 sec 1 min/kb	5
Regular Cycling	95°C Annealing Temperature[2] 72°C	30 sec 30 sec 1 min/kb	25
Final Extension	72°C	5 min	1

[2]*Annealing temperature is calculated based on your primers*

4. After PCR, maintain the reaction at 4°C or store at -20°C until use.
5. Analyze the amplification products by agarose gel electrophoresis.

General Notes

- Adjust the annealing temperatures accordingly depending on primer T_M.
- Start with high-quality, purified DNA templates to achieve even greater PCR success.

Applied Biological Materials Inc. • 1-866-757-2414 • info@abmGood.com • www.abmGood.com