

SapphireAmp® Fast PCR Master Mix (Cat. # RR350A/B)

Protocol At-a-Glance: Colony PCR with SapphireAmp Fast PCR Master Mix

SapphireAmp Fast PCR Master Mix is a 2X PCR premix containing a hot start PCR enzyme, dNTPs, and buffer optimized for high-speed PCR. The mix also contains a blue loading dye that enables reactions to be directly loaded on an agarose gel for electrophoresis.

The SapphireAmp premix is ideal for colony PCR-based screening for inserts <6 kb. To perform colony PCR using SapphireAmp Fast:

1. Prepare the following reaction mixture for PCR (50 µl reaction volume):

Component	Amount
SapphireAmp Fast PCR Master Mix	25 µl
Forward Primer	0.2 µM (final conc.)
Reverse Primer	0.2 µM (final conc.)
dH ₂ O	to 50 µl

2. Dispense the reaction mixture into PCR tubes.
3. Touch an individual colony with a sterile pipette tip, touch the tip to a new plate (selective media) to create a replica plate, and mix the remaining cells on the tip with the reaction mixture.
4. Transfer the reactions to a thermal cycler and perform PCR according to the following cycling conditions:

Initial denaturation:

94°C, 1 min



Amplification:

98°C 5 sec

55°C 5 sec

72°C 10 sec/kb*

30 cycles

* a 5 sec. extension is recommended for amplicons less than 1 kb

5. To analyze PCR products by gel electrophoresis, load 5 µl of the PCR reaction onto a 1% agarose gel for electrophoresis. The dye runs at ~1 kb and 3-5 kb.