



KIAGENE FANAVAR

## 2X Syber Master Mix

Cat. No:

FPLF009.0500

FPLF009.1000

FPLF009.2000

Contents:

Components	25 rxn	50 rxn	100 rxn
2X Syber Master Mix	0.5 ml	1ml	2ml
Passive Reference Dye (50X ROX Dye)	25 µl	50 µl	100 µl

### Kit storage:

⚠ This kit should be stored at -20 °C.

If properly stored, all kit components are stable until the expiration date printed on the label.

### Additional Equipment and Reagent required

- Template: cDNA
- Forward and reverse primers
- 0.2 or 0.5-mL nuclease-free microcentrifuge tubes

### Application

Kiagene SYBR Green PCR Master Mix provides maximum flexibility at reduced cost because no target-specific TaqMan probes are required. SYBR Green dye is a double-stranded DNA binding dye that detects any double-stranded DNA generated during PCR. The hot-start enzyme DNA Polymerase minimizes nonspecific product formation (including primer-dimers), yielding superior performance and sensitivity. Passive Internal Reference is provided to normalize non-PCR-related fluorescence fluctuations. This minimizes well-to-well variability that can result from a variety of causes, such as pipetting error or sample evaporation. SYBR Green dye is ideal for target identification (screening assays) or when a limited number of assays is needed.

### Handling Requirements and Safety Information

- ⚠ use RNase-free and DNase-free materials
- ⚠ Do not use any modified Protocols.
- ⚠ Do not pool reagents from different lot numbers.
- ⚠ Immediately after usage, close all bottles in order to avoid leakage, varying buffer concentrations or buffer conditions.
- ⚠ After first opening store all bottles in an upright position.
- ⚠ Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.
- ⚠ Do not contaminate the reagents with bacteria, virus, or nucleases. Use disposable pipets and nuclease free pipet tips only, to remove aliquots from reagent bottles.

### Protocol

1. Prepare reaction by adding the following components in the order listed in the following table.

Components	20 µl rxn	50 µl rxn	Final conc.
2x Syber Master Mix	10 µl	25 µl	1x
Passive Reference Dye (50X ROX Dye)	0.4µl	1µl	1x
Forward Primer	x µl		0.5 µM
Reverse Primer	x µl		0.5 µM
Template DNA	x µl 5–100 ng cDNA		
nuclease free Water	Add to 20 µl	add to 50 µl	-

⚠ 1 µM (µMolar) = 1 pmol/µl (pico moles/µl).

2. Run a Real-Time PCR machine program set to the following parameters according to the protocol to be performed.

Cycle	Time	Temp °C
1	10 min	95
30	15 sec	95
	30 sec	50-60
	30-60 sec	72
1	5 min	72

6. Perform a melting curve analysis of the PCR product(s).