

## SYBR HS-qPCR Master Mix (Hi-ROX), 2X

(With Hot Start Taq DNA Polymerase)



MM2173



100 TESTS



Wet or Dry Ice



Store at: -20°C

### Components

Kit Contents	Quantity/ Volume
SYBR HS-qPCR Master Mix (Hi-ROX), 2X	1.25 ml

\* Not more than 50 Freeze-Thawing cycles

### Description

SYBR HS-qPCR Master Mix (Hi-ROX), 2x is developed for quantitative real-time PCR with fluorescent dye SYBR Green I on PCR platforms that use fluorescent dye ROX as a reference. It includes all of components necessary to PCR (**highly processive recombinant Hot Start "HS" Taq DNA polymerase, deoxynucleoside triphosphate mix, PCR buffer, Mg<sup>2+</sup>, SYBR Green I, fluorescent dye ROX plus Inert dye** except for DNA template and primers). The mix is optimized for conducting consistent and efficient real-time Hot start PCR of genomic, plasmid and viral DNA samples. The solution includes substances increasing half-life and processivity of HS-Ta<sub>q</sub> DNA polymerase by enhancing its stability during PCR.

SYBR HS-qPCR Master Mix, 2x contains components that influence primer annealing temperature and characteristics of template melting, thus enabling to increase the specificity of PCR and use templates with complicated partial structure.

The DNA polymerase included in the mix, is inactive at room temperature and its activation requires preheating at 95 °C for 5 min.

(SMG-036-01/00) (1)



### SYBR HS-qPCR Master Mix, 2x contains

100mM Tris-HCl (pH 9.1 at 25°C), 150mM KCl, 0.4mM of each deoxynucleoside triphosphate, 5mM MgCl<sub>2</sub>, 0.12U/μl Taq DNA polymerase, 0.025% Tween20, stabilizers of HS-Ta<sub>q</sub> DNA polymerase, SYBR Green 1, 1μM fluorescent dye ROX and inert dye.

### Applications

1. Real-Time PCR with intercalating dye SYBR Green I, 2. Conventional PCR
3. High-throughput PCR, 4. Genotyping

### HS- Taq DNA Polymerase features

Recombinant HS-Ta<sub>q</sub> DNA polymerase has 5'→3'DNA-dependent polymerase activity and 5'→3' exonuclease activity.

### SYBR Green I

SBRY Green I is a fluorescent intercalating dye for quantitative and qualitative detection of PCR products during real-time PCR. SYBR Green I provide easy and economical way for detection and quantitative assessment of PCR products during real-time PCR without a need for specific fluorescent probes. During amplification, SYBR Green I dye penetrates into the minor groove of DNA products and emits stronger fluorescent signal than unbound dye. Absorption and emission maxima of SYBR Green I are 494nm and 521nm, respectively, which enables to use it for every real-time PCR platform existing to date.

### Passive reference dye ROX

The mix includes passive fluorescent dye ROX, which serves as the inner reference for SYBR Green I signal normalization when using PCR platforms supporting such function. ROX allows adjusting variations between tubes that occur due to the pipetting errors and fluctuation in fluorescence. The presence of ROX does not affect the course of PCR and shift in fluorescence signal in case if the mix is used with other PCR platforms. However, it should be taken into account that the presence of ROX fluorophore restricts its use for oligonucleotide prob, as well as for other dyes that share similar spectral characteristics (Em ~ 621 nm).

### Inert dye

The blue dye is an inert dye and won't affect qPCR efficacy at all. The added dye may ease the monitoring of liquid handling and pipetting.

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### Benefits of use

1. The enzyme with hot start capability increases reaction specificity and sensitivity
2. HS-Taq DNA polymerase activation requires not more than 5 min heating
3. High selectivity and reaction yield
4. The mix is colored for easy pipetting
5. Reduced preparation time
6. Low contamination risk when mixing PCR components
7. Standardized conditions of the same-type reactions (reduced pipetting error during mixing PCR components in a series of experiments)
8. Provides data normalization

### Limits of use

Not recommended to use for real-time PCR with fluorescently labeled probes.

### Recommended qPCR reaction mix

1. Defrost the reaction mixture and stir thoroughly.
2. Put thin-wall PCR tubes on ice and add the following components considering the final volume of a reaction mixture equal to 25  $\mu$ l:

Component	Volume	Final concentration
SYBR HS-qPCR Master Mix (Hi-ROX), 2x	12.5	1X
Forward primer	variable	0.1 – 600nM
Reverse primer	variable	0.1 – 600nM
DNA template	Variable	1pg – 1 $\mu$ g
Sterile water	up to 25 $\mu$ l	-






### Recommended qPCR cycles

Step	Temperature, °C	Incubation time	Number of cycles
Preliminary denaturation	95	5 min	1
Denaturation	95	5-15 sec	30- 50
Annealing	50- 68	5-15 sec	
Elongation	58- 72	10-30 sec	
Melting curve (recommended)	65 - 95	-	1

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**Note:** Monitoring of real-time PCR can be conducted at 72°C in case of absence of non-specific products (primer dimers). In case if non-specific products are formed with Tm1 lower than Tm2 of the target product. Monitoring should be performed at the temperatures between Tm<sub>1</sub> and Tm<sub>2</sub>.

### Signs

Signs	Definitions	Signs	Definitions
	Product shipping conditions		Name and address of the manufacturer of the product
	For Research Use Only		Product technical code
	Number of usable tests		

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