

SYBR HS-qPCR Master Mix (No-ROX), 2X

(With Hot Start Taq DNA Polymerase)

REF

MM2175



100 TESTS



Wet or Dry Ice



Store at: -20°C

Components (supplied)

Contents of the kit	Amounts
SYBR HS-qPCR Master Mix (No-Rox), 2x	1.25 ml

Description

SYBR HS-qPCR Master Mix, 2x is developed for quantitative real-time PCR with fluorescent dye SYBR Green I. It includes all of components necessary to PCR (highly processive recombinant Hot Start "HS" Taq DNA polymerase, deoxynucleoside triphosphate mix, PCR buffer, Mg²⁺, SYBR Green I 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-Taq 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.

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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 MgCl₂, 0.12U/μl Taq DNA polymerase, 0.025% Tween20, stabilizers of HS-Taq DNA polymerase, SYBR Green 1 and inert dye.

Applications

- Real-Time PCR with intercalating dye SYBR Green I
- Conventional PCR
- High-throughput PCR
- Genotyping

HS- Taq DNA Polymerase features

Recombinant HS-Taq 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.

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.

Benefits of use

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

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 µl:

Component	Volume	Final concentration
SYBR HS-qPCR Master Mix, 2x	12.5	1X
Forward primer	variable	0.1 – 600 nM
Reverse primer	variable	0.1 – 600 nM
DNA template	Variable	1 pg – 1 µg
Sterile water	up to 25µl	-

Recommended qPCR cycles:

Step	Temperature °C	Incubation time	Number of cycles
Preliminary denaturation	95	5-7 min	1
Denaturation	95	15 sec	25- 50
Annealing	50- 68	10-30 sec	
Elongation	58- 72	30-60 sec	
Melting curve (recommended)	65 - 95	--	1

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 T_{m1} lower than T_{m2} of the target product. Monitoring should be performed at the temperatures between T_{m1} and T_{m2} .



Unit 9, Rouyesh building, Science and Technology Park, Tarbiat Modares University, Pajouhesh Blvd, Tehran, Iran



+982191082111



hi@sinaclon.com



www.sinaclon.com