SinaProbe HS-qPCR Hi-ROX Mix, 2X

For Research Use Only

Cat. No.: MM2161 **Quantity:**100 reactions/ 25µl

Store at:-20°C (not more than 50thawing-freezing cycles) **Shipment:** Wet or dry Ice

Description: SinaProbe HS-qPCR Hi-ROX Mix,(2x) is developed for quantitative real-time PCR with fluorescent probes on PCR platforms that use ROX as a reference dye. SinaProbe HS-qPCR Hi-ROX Mix,2x includes all the components necessary for PCR (**Highly processive recombinant HS-*Taq* DNA polymerase**, **Deoxynucleoside triphosphate mix**, **PCR buffer**, **Mg2+**, **ROX dye** except for DNA template, primers and probe). Additionally, sterile and PCR grade water is supplied.The mix is optimized for consistent and efficient real-time hot-start PCR of genomic, plasmid and viral DNA samples. The mix is supplemented with additives that increase half-life and processivity of HS-Taq DNA polymerase by enhancing its stability during PCR. SinaProbe HS-qPCR Hi-ROX Mix,2x does not contain substances affecting primer annealing temperature and characteristics of template melting.DNA polymerase included in the SinaProbe HS-qPCR Hi-ROX,2x is inactive at room temperature, and its activation requires preheating of the reaction solution at 95˚C for 5 min. The master mix is ideally suitable for PCR platforms use ROX passive dye as a reference guide: Life Technologies (ABI) 7000, 7300, 7700, 7900HT, StepOne Plus.

Components (supplied):

SinaProbe HS-qPCR Hi-ROX Mix, 2x	1250 µl
Distilled water	1000 µl

SinaProbeHS-qPCR Hi-ROX Mix, 2x contains:

100 mMTris-HCl (pH 8.5 at 25 ˚C), 100 mMKCl, 0.4 mM each deoxynucleoside triphosphate, 10 mM $MgCl_2$, 0.1 U/µl HS-*Taq* DNA polymerase, 0.025% Tween 20, stabilizers of HS-*Taq* DNA polymerase, 0.9 µM ROX fluorescent dye.

Applications:

- Real-time hot start PCR with fluorescently labeled probes and ROX as a reference dye
- Conventional PCR
- High-throughput PCR
- Multiplex PCR
- Genotyping

HS- *Taq*DNA Polymerase features

Recombinant HS-*Taq*DNA polymerase has 5'→3' DNA-dependent polymerase activity and 5'→3' exonuclease activity.

Passive fluorescent ROW dye

The mix includes passive fluorescent ROX dye, which serves as the inner reference for signal normalization of dyes comprising oligonucleotide probes when using PCR platform with such function (Applied Bio systems).ROX allows adjustment of variation between tubes (wells) that occur due to the pipetting errors and fluorescence fluctuation. 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 probes, as well as for other dyes that share similar spectral characteristics(EM ~ 621Nm).

Reaction mix features

●The mix is optimized for real-time hot-start PCR with fluorescently labeled probes
●Allow normalization to ROX reference dye
●prevents re-amplification of extraneous PCR products

Benefits of use

- The enzyme with hot start capability enhances reaction specificity
- Activation of HS-*Taq* DNA polymerase requires not more than 5 min heating
- High selectivity and reaction yield
- Reduced preparation time
- Low chance of contamination during preparation of PCR solution
- Possibility of data normalization
- Standardized conditions of the same-type reactions (reduce pipetting error during mixing PCR components in a series of experiments)
- Minimized efforts

Limits of use

Not recommended to use for real-time PCR with intercalating dyes.

Recommended qPCR reaction mix:

1. Unfreeze the reaction mixture and stir gently.
2.Add the following components into thin-well PCR tubes considering the final volume of a reaction mixture equal to 25µl:

Component	Volume	Finalconcentration
SinaProbe HS-qPCR Hi-ROX Mix, 2x	12.5	1×
Forward primer	variable	0.1–600nM
Reverse primer	variable	0.1–600nM
Probe	variable	0.1–300nM
DNAtemplate	Variable	1 pg–1µg
Sterile water	up to25µl	

Recommended qPCR cycles:

Step	Temperature,°C	Incubation time	Number of cycles
Preliminary denaturation	95	5-7min	1
Denaturation	95	15sec	30-50
Annealing	50- 68	10-30sec	
Elongation	58- 72	30-60sec	

Or:

Step	Temperature,°C	Incubation time	Number of cycles
Preliminary denaturation	95	5-7min	1
Denaturation	95	15sec	30- 50
Annealing /Elongation	50- 68	1 min	

Storage terms: 1year(under proper storage and transportation conditions).

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