Probe HS-qPCR Master Mix (Hi-Rox), 2X

REF **MM2162**

1250µl/100 reaction

Wet or Dry Ice

RUO **Store at: -20°C**

(Not more than 50thawing-freezing cycles)

Component

Probe HS-qPCR Master Mix, 2X	**1250µl**

Description

Probe HS-qPCR Master Mix, (2x) is developed for quantitative real-time PCR with fluorescent probes. It includes all the components necessary for PCR (Highly processive recombinant HS-Taq DNA polymerase, Deoxy nucleoside triphosphate mix, PCR buffer, Mg^{2+}, ROX dye except for DNA template, primers and probe). The mix is optimized for consistent and efficient real-time hot-start PCR of genomic, plasmid and viral DNA samples. It is supplemented with additives that increase half-life and processivity of HS-Taq DNA polymerase by enhancing its stability during PCR. Probe HS-qPCR Master Mix, 2x does not contain substances affecting primer annealing temperature and characteristics of template melting. DNA polymerase included in the Probe HS-qPCR Master Mix, 2x is inactive at room temperature, and its activation requires preheating of the reaction solution at 95℃ for 5min. **The master mix is ideally compatible for all PCR platforms/devises as well as platform using ROX passive dye as a reference guide: Life Technologies (ABI) 7000, 7300, 7700, 7900HT, StepOne Plus.**

Probe HS-qPCR Master Mix, 2x contains:

100mM Tris-HCl (pH 9.1 at 25 C), 150mM KCl, 0.4mM each deoxy nucleoside triphosphate, 6mM MgCl2, 0.1U/µl HS-Taq DNA polymerase, 0.025% Tween 20, stabilizers of HS-Taq DNA polymerase, 1µM ROX fluorescent dye.

Applications

- Real-time PCR with fluorescently labeled probes
- Conventional PCR
- High-throughput PCR
- Multiplex PCR
- Genotyping

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
- 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.

Reaction mixes features

- Recombinant HS-Taq DNA polymerase has 5'→3' DNA-dependent polymerase activity and 5'→3' exonuclease activity.
- Allow normalization to ROX reference dye (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).
- prevents re-amplification of extraneous PCR products

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	Final concentration
Probe HS-qPCR Master Mix, 2x	12.5	1X
Forward primer	variable	0.1 – 600nM
Reverse primer	variable	0.1 – 600nM
Probe	variable	0.1–300nM
DNA template	Variable	1pg – 1µg
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**	

Or:

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

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