gb HRM PCR Master Mix

Description of the product

gb HRM PCR Master Mix contains hot-start Taq DNA polymerase, reaction buffer, dNTP, $MgCl_2$, fluorescent dye (with detection in FAM/Sybr channel) and other components needed for PCR amplification with subsequent HRM analysis. For mixing of reaction mixture only add primers and template.

gb PCR Master Mix by application	gb HRM
end-point PCR, common PCR amplification	
real-time PCR without probes	✓
real-time PCR with hydrolysis probes	
real-time PCR with LNA probes	
real-time PCR with hybridization probes	
real-time PCR with High Resolution Melting Analysis	✓
real-time PCR with low DNA samples	
PCR/real-time PCR with inhibited samples	✓

Purpose of the product

gb HRM PCR Master Mix is intended for amplification of DNA with subsequent High Resolution Melting (HRM) analysis. HRM analysis is an efficient melt curve analysis method for genotyping, mutation scanning prior to sequencing analysis, DNA mapping, species identification or DNA methylation analysis etc. It is based on dissociation behavior of nucleic acids due to increasing temperature gradient. With the use of a double stranded DNA fluorescent dye it allows to detect even a single base change in the sequence of an amplified PCR product. No additonal instrumentation is required; HRM analysis can be performed on any real-time PCR cycler simply by adding a Melt Curve step analysis with high resolution directly after a PCR amplification.

Available products

Cat. No.	Product	rxn
3017	gb HRM PCR Master Mix	100

1 tube contains reagents to provide 100 PCR reactions (20 µl volume of each reaction).

Parameters of the product

- **Master Mix is 2x concentrated.**
- **One vial contains 1 ml of Master Mix** (this amount is sufficient for 100 reactions of 20 µl).
- **PCR Master Mix** contains hot-start *Taq* DNA polymerase, reaction buffer, dNTP, $MgCl_2$, fluorescent dye (with detection in FAM/Sybr channel) and other components needed for PCR amplification with subsequent HRM analysis.
- **PCR Master Mix** does not contain any passive reference dye.
- If the real-time PCR cycler requires normalization with a passive reference dye, it can be added to the reaction mixture separately (*see Passive Reference Dye, Cat. No. 3010 – on request*)

Amplification protocol

Step	Temperature	Time	Cycle number
Initial denaturation	95 °C	3 min	
Denaturation	95 °C	5 s	30 - 50
Annealing + elongation (+ flurescence acquiring)	55 - 65 °C	10 s	
Extension	72 °C	20 s	
Denaturation	95 °C	2 min	
HRM analysis / Melt Curve (+ flurescence acquiring)	50 °C	20 s	
	70 - 95 °C	5 s	rise by 0,1 - 0,2 °C