EURx® MOLECULAR BIOLOGY PRODUCTS

HRM PCR Master Mix (2x)

Kit Components

Component	Cat. No. E0440-01 100 reactions of 25 µl	Cat. No. E0440-02 500 reactions of 25 µl
HRM PCR Master Mix (2x)	1 x 1.25 ml	5 x 1.25 ml
Water, nuclease free	1 x 1.25 ml	5 x 1.25 ml

Storage

Store at -20°C in the dark for long-term storage or at 4°C for up to 1 month.

Description

- HRM PCR Master Mix (2x) is designed for high-resolution melting analysis of DNA samples.
- HRM PCR Master Mix (2x) enables detection of gene mutations and SNPs (even the most challenging sequence differences, class III and IV SNP).
- The master mix contains onTaq DNA Polymerase, optimized reaction buffer, dNTPs (dATP, dCTP, dGTP, dTTP of ultrapure quality) and fluorescent dye.
- onTaq DNA Polymerase is a modified "hot start" enzyme which provides very tight inhibition of the polymerase activity at moderate temperatures and allows room temperature reaction setup.
- The polymerase activity is restored during the initial denaturation step when amplification reactions are heated at 95°C for 15 minutes.
- Use of the "hot start" enzyme prevents extension of misprimed products and primer-dimers during reaction setup leading to higher specificity and sensitivity of PCR reactions.
- The fluorescent dye selectively binds all double-stranded DNA molecules and emits a fluorescent signal on binding. The spectral properties of the dye is similar to those of SYBR Green I. The excitation and emission maxima are at 500 nm and 530 nm, respectively. This allows detection on channels preset for SYBR Green detection.
- In contrast to SYBR Green, the dye can be used in higher concentrations, allowing efficient inhibition-free PCR and HRM analysis.

Protocol

Preparation of PCR Reaction:

Component	Volume/reaction	Final concentration
HRM PCR Master Mix (2x)	12.5 µl	1 x 2.5 mM $MgCl_2$
Forward Primer	Variable	0.3 µM
Reverse Primer	Variable	0.3 µM
Template DNA	Variable	Eukaryotic: 1-50 ng DNA/reaction Microbial: 1-50 pg DNA/reaction
Water, nuclease free	To 25 µl	-
Total volume	25 µl	-

Notes:

1. Minimize exposure of HRM PCR Master Mix (2x) to light during handling to avoid loss of fluorescent signal intensity.
2. A reaction volume of 25 µl should be used with most real-time cyclers. Other reaction volumes may be used if recommended for a specific instrument.
3. Design assays with PCR product lengths of 70-250 bp. For SNP analysis optimal amplicon length is 70-150 bp. Larger products usually provide lower resolution.
4. Thaw and mix well all reaction solutions. HRM PCR Master Mix (2x) should be warmed up to room temperature and thoroughly mixed by vortexing. Dye precipitation may occur during storage the master mix at –20°C for a long time and the orange pellet may be seen at the bottom of the tube.
5. Set up PCR reactions at room temperature. Use of HRM PCR Master Mix (2x) allows room temperature reaction setup.
6. Prepare a reaction master mix by adding all the reaction components except template DNA.
7. Mix the reaction mix thoroughly and dispense appropriate volumes into PCR tubes or plates.
8. Add equal amounts and volumes of template DNA to the individual PCR tubes or wells containing the reaction mix. DNA samples should be quantified and adjusted to the same concentration. C_T values should be below 30 and differ by no more than 3 C_T values.
9. Centrifuge briefly to settle down the reaction components and remove bubbles. Bubbles interfere with fluorescent detection.
10. Place the samples in the real-time cycler and start the PCR program, followed by HRM analysis.
11. A final primer concentration of 0.3 µM is usually optimal for HRM analysis using Eurx HRM PCR Master Mix (2x), but can be individually optimized in range of 0.2 µM to 0.8 µM. The recommended starting concentration is 0.3 µM. Raising primer concentration may increase PCR efficiency, but negatively affect PCR specificity.

Thermal Cycling Conditions:

Step	Temperature	Time	Number of Cycles
Initial Denaturation	95°C	15 min	1
Denaturation Annealing/Extension	95°C 60°C	10 s 30 s	40-45
HRM	65-95°C		1
Cooling	4°C	Indefinite	1

Notes:

1. onTaq DNA Polymerase requires 15 min incubation at 95°C to be activated.
2. Check the real-time cycler's user manual for correct instrument setup. Set the ramp rate, temperature increment in seconds for HRM analysis according to manufacturer's recommendation.
3. Use sufficient PCR cycles so that all samples have reached the plateau phase of PCR. The amount of DNA affects the melting temperature of the PCR product.
4. It is recommended to initially determine the melting point for each new HRM PCR product. Run HRM analysis in temperature range from 65°-95°C. After determination of Tm, you may run HRM from 5°C below the lowest Tm to 5°C above the highest Tm of all PCR products.
5. Data acquisition during PCR amplification should be performed during the combined annealing/extension step.
6. Check the PCR product specificity by gel electrophoresis when designing a new assay.