TIARIS™ HRM FAST PCR Mastermix (2X)

Ordering Info

TBK0076, 100 reactions
TBK0077, 500 reactions

Description

High Resolution Melt (HRM) analysis is a post-PCR technique based on the precise monitoring of double-stranded DNA dissociation through gradual temperature increases. It uses intercalating fluorescent dyes that emit signal only when bound to double-strand DNA, allowing the detection of subtle sequence differences through changes in melting profiles. Due to its high sensitivity, HRM has become a powerful tool in several applications, such as SNP genotyping, detection of somatic mutations, and DNA methylation analysis.

The TIARIS™ HRM FAST PCR Mastermix (2X) has been specifically optimized for high-resolution melting applications, offering excellent performance in terms of amplification efficiency, signal consistency, and mutation discrimination. The mix combines a highly efficient enzyme with an optimized buffer system, enabling robust amplification of both GC-rich and AT-rich templates under standard and fast qPCR cycling conditions. The intercalating dye used in this mastermix does not inhibit PCR, allowing for exceptional sensitivity and specificity, while also preventing the formation of primer-dimers and non-specific products.

Features

- Ready to use.
- Accurate distinction of SNP classes I-IV: TIARIS™ HRM FAST PCR Mastermix (2X) can distinguish all types of SNPs. These include:

  Class I: C to T and G to A
  Class II: C to A and G to T
  Class III: C to G
  Class IV: A to T

- Compatible with fast and standard PCR program.

Kit Components

Components	TBK0076	TBK0077
TIARIS™ HRM FAST PCR Mastermix (2X)	1mL	5x 1mL
PCR Grade Water	2 mL	2x 2mL
ROX reference dye	1 vial	1 vial

Order Info Kit Components: ROX Reference Dye (TBR0278) | PCR Grade Water, nuclease free (TBB0303).

Storage

Shipped on blue ice. Upon receipt, store kit components immediately at -20 °C. Avoid prolonged exposure to light and repeated freeze-thaw cycles.

Instrument Compatibility

Compatible with real-time PCR instruments that support HRM analysis, including Bio-Rad CFX, Roche LightCycler, QuantStudio, Applied Biosystems 7500 Fast, etc.

Applications

- SNP genotyping
- Detection of insertions, deletions and translocations
- DNA methylation analysis
- Gene scanning

Technical Assistance

Please refer any technical questions to support@tiarisbiosciences.com

PROTOCOL

1. Gently vortex and briefly centrifuge kit components after thawing.
2. Place a tube on ice and add the following components for each 20 µL reaction. Prepare sufficient master mix for the number of reactions. Consider one or two extras:

Components	Volume	Final Concentration
TIARIS™ HRM FAST PCR Mastermix (2X)	10 µL	1x
Forward primer (10 pmol/µL) *	0.8 µL	400 nM
Reverse primer (10 pmol/µL) *	0.8 µL	400 nM
Template DNA (step 5) **	-	-
PCR Grade Water	up 20 µL	
Final Volume	**20 µL**	

** Optimal amplicon length should be 80-200 bp, and should not exceed 400 bp. For SNP analysis, use of PCR products of 80–150 bp is recommended.*
*** Since the HRM analysis is extremely sensitive, please use the same template amount between different reactions. Optimal amounts of template: Use 1 ng to 50 ng of template genomic DNA.*

3. Dispense the master mix into wells of PCR plate.
4. Gently vortex and spin down the samples.
5. Add the DNA sample to each well. Mix thoroughly by pipetting.
6. Seal the PCR plate with an optical adhesive film.
7. Set-up the qPCR cycling program (if applicable, select fast mode on the instrument):

Suggested thermal cycling conditions:

Process	Cycles	Temperature	Time
Enzyme Activation	1 x	95 °C	2-3 min
Denaturation	40 x	95 °C	5 sec
Annealing/ Extension		60-65°C	20-30sec*
HRM analysis: *After the reaction has reached completion, refer to the instrument instructions for the option of melt-profile analysis.*			

**Select the shortest time possible but not less than 20 sec and do not exceed 30 seconds.*
In case of 3-step cycling, anneal at optimal annealing temperature for 20 sec and minimum time necessary at 72°C for data acquisition (according to manufacturer´s guidelines).

Note: This Kit includes ROX Reference Dye, which helps eliminate background signal interference and correct fluorescence signal variation between wells.

The PCR Master Mix does not contain any passive reference dye. If your real-time PCR instrument requires normalization using a passive reference dye, you may add ROX Reference Dye to the Master Mix prior to first use. Depending on your equipment, add 2 µL (Low ROX) or 20 µL (High ROX) of the ROX reference dye to 1 mL **TIARIS™ HRM FAST PCR Mastermix (2X)** and vortex briefly to mix. Once ROX has been added, the Master Mix can be used directly or stored at -20°C for up to 1 year.