TIARIS™ SNP PCR Mastermix (2X)

Ordering Info

TBK0079, 100 reactions
TBK0080, 500 reactions

Description

The TIARIS™ SNP PCR Mastermix (2X) is based on a highly specific engineered Taq DNA polymerase variant and an optimized buffer system, both of which enable reliable and accurate allelic discrimination.

The modified Taq polymerase included in the mix is an aptamer-inhibited hot-start enzyme, specifically designed for applications requiring high single-nucleotide discrimination — for instance, in allele-specific amplifications (ASA) by PCR, primer extension assays, methylation-specific PCR (MSP), HLA genotyping, or the analysis of single CpG methylation sites. The enzyme incorporates a special N-terminal deletion and proprietary amino acid substitutions within its active site. These modifications increase its sensitivity to mismatches at the 3' end of primers, significantly enhancing specificity.

As a result, non-specific amplicons are minimized when primers are not perfectly matched to the target sequence. Additionally, Taq DNA Polymerase shows 5'-3', especially applicable for usage together with hydrolysis probes.

Applications

- SNP-detection by allele-specific amplification (ASA) / Allele-specific PCR.
- Quantification of mutations.
- Monitoring, verification and detection of point mutations.
- Methylation specific PCRs (MSP) after bisulfite treated DNA (CpG methylation sides)
- HLA genotyping.
- Real time PCR with hydrolysis probes.
- Direct PCR.

Kit Components

Components	TBK0079	TBK0080
TIARIS™ SNP PCR Mastermix (2X)	1.25 mL	5x 1.25 mL
PCR Grade Water	2 mL	2x 2mL

Order Info Kit Components: *PCR Grade Water, nuclease free (TBB0303).*

Storage

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

Features

- Ready-to-use format. The 2X Master Mix contains all components necessary for PCR-based genotyping, including an engineered DNA polymerase, an optimized reaction buffer, and ultrapure dNTPs. Only target-specific primers and template DNA need to be added.
- Hot-start enzyme formulation prevents non-specific amplification and increases assay specificity.
- Modified Taq DNA polymerase. Modifications increase its sensitivity to mismatches at the 3' end of primers, significantly enhancing specificity.
- 5'→3' exonuclease activity, making the enzyme especially suitable for use with hydrolysis probes such as TaqMan™ probes.

Technical Assistance

Please refer any technical questions to support@tiarisbiosciences.com

PROTOCOL

This protocol serves as a guideline for PCR amplification. Optimal reaction conditions such as incubation times, temperatures, and amount of template DNA may vary and must be determined individually.

1. Gently vortex and briefly centrifuge kit components after thawing.
2. Place a tube on ice and add the following components for each 25 µL reaction. Prepare sufficient master mix for the number of reactions. Consider one or two extras:

Components	Volume	Final Concentration
TIARIS™ SNP PCR Mastermix (2X)	12.5 µL	1x
Forward primer (10 pmol/µL) *	0.5 µL	200 nM
Reverse primer (10 pmol/µL) *	0.5 µL	200 nM
Template DNA (step 5) **	-	-
PCR Grade Water	up 25 µL	
Final Volume	25 µL	

** Primers should ideally have a GC content of 40-60%. Master Mix is optimized for short amplicon length (about 60 – 200bp), but also longer amplicons are possible. The addition of additional MgCl2 (0.5 – 1.5mM) might be needed in case of longer amplicons (>500bp).*
*** <10ng plasmid DNA or <500ng 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:

Suggested thermal cycling conditions:

Process	Cycles	Temperature	Time
Enzyme Activation	1 x	95 °C	2 min
Denaturation	25-40 x	95 °C	15 sec
Annealing		>55°C*	10 sec
Extension		72°C**	30 sec/250bp

** The Taq Polymerase is an aptamer inhibited DNA polymerase. The aptamer oligo used reversibly inhibits the polymerase at temperatures <55°C! Therefore, the primers should ideally have an annealing temperature of >57°C. Please design your primers accordingly.*

*** Approx. 0.5kbp per minute extension rate*