Precision Melt Supermix

100 x 20 µl reactions (Catalog #172-5110) 1,000 x 20 µl reactions (Catalog #172-5112)

For research purposes only. Store at –20°C and protect from light.

Storage and Stability
Precision melt supermix is stable until the date indicated when stored in a constant temperature freezer at –20°C. For convenience, it may be refrozen for longer-term storage.

Kit Contents
Precision melt supermix is a 2x concentrated, ready-to-use real-time PCR reagent optimized for specificity and post-PCR high resolution melt (HRM) analysis. It contains hot-start iTaq™ DNA polymerase, dNTPs, $MgCl_2$, EvaGreen dye, enhancers and stabilizers in a proprietary formulation optimized for HRM applications (SNP genotyping, mutation scanning, and methylation detection).

iTaq DNA polymerase is an antibody-bound hot-start Taq enzyme that ensures highly specific PCR amplification of target DNA. iTaq DNA polymerase activity is inhibited at room temperature but is fully activated at 95°C for 2 minutes. This prevents the formation of nonspecific priming during reaction setup and ensures highly specific amplification, which is essential for accurate melt curve analysis.

EvaGreen dye is a fluorescent dye that binds specifically to double-stranded DNA. It is used to monitor the PCR amplification and the post-PCR melting processes. The dye generates an increased fluorescent signal once bound to double-stranded DNA and the fluorescent signal decreases when dsDNA is melted into single-strand DNA. A saturating concentration of dye is used in the supermix to prevent dye molecule redistribution during melting and to provide superior HRM resolution.

Guidelines for Use

1. Thaw precision melt supermix and other reaction components. Mix each component thoroughly and centrifuge briefly to collect at the bottom of the tube.

2. Prepare an adequate volume of assay master mix by adding all required components except the sample template according to the table below.

Table 1. Reaction setup

Component	Volume per 20 µl Reaction, µl	Volume per 10 µl Reaction, µl	Final Concentration
Precision melt supermix	10	5	1x
2 µM (each) primer mix	2	1	200 nM (each primer)
DNase-free water	3	--	--
Assay master mix volume	**15**	**6**	
Genomic DNA* (add in step 4)	5	4	1 to 50 ng / reaction**
Total reaction mix volume	**20**	**10**	**--**

* DNA sample concentration should be normalized so that equal volume of DNA is added for all reactions.
** For other types of sample DNA, adjust the amount so that all reactions give a Cq value less than 30.

3. Mix the assay master mix thoroughly and dispense equal aliquots into each PCR tube or well of a PCR plate.

4. Add equal amounts of sample DNA (1 to 50 ng genomic DNA, in the same volume) to each PCR tube or wells of a plate, seal, and vortex for 20 to 30 sec to ensure thorough mixing of reactions. Centrifuge the tubes or plate to remove air bubbles.

   Notes:
   - Use the same DNA preparation method for all samples. This will minimize salt or buffer variation between samples and subsequent reactions.
   - Ensure good mixing to reduce variability, especially when the assay master mix is made before dispensing into tubes or wells. Salt or buffer variation between reactions may affect melt profile reproducibility and genotyping accuracy.

5. Follow the PCR and HRM guidelines in Table 2. For CFX instruments, select SYBR/FAM only Scan Mode and SYBR as the fluorophore in plate setup. For other real-time cyclers, refer to the appropriate instruction manual for guidelines.

Notes:

- For SNP genotyping assays with amplicon sizes <200 base pairs (bp), a 2-step PCR cycling protocol works well. For difficult amplicons or for gene mutation scanning assays with larger amplicons (>200 bp), a 3-step PCR cycling protocol is recommended to improve PCR performance.
- Precision melt supermix and the PCR/HRM cycling protocols have been optimized for assays with a primer Tm = 60°C designed using the open source Primer3 program (http://frodo.wi.mit.edu/) under its default Tm calculation parameter settings. For non-Primer3 designed assays, use the Primer3 or its derivative program (e.g. the UCSC In-Silico PCR program http://genome.ucsc.edu/cgi-bin/hgPcr?command=start) to calculate the primer Tm, and use the calculated Tm for the PCR annealing temperature.
- To achieve optimal SNP discrimination, design assays with an amplicon size between 70 to 200 bp. For genotyping class III (G>C) or class IV (A>T) SNPs, design assays with amplicon sizes <100 bp.

Table 2. Optimized PCR and HRM protocol for precision melt supermix on Bio-Rad's CFX96™ and CFX384™ systems.

Cycling Step	Temperature	Time	# Cycles	Additional Comments
Initial DNA denaturation	95°C	2 min	1	Complete activation of hot-start polymerase.
2-step or 3-step PCR Cycling				
Denaturation	95°C	10 sec	40–45	A 3-step run protocol is recommended for amplicons >200 bp or GC-rich targets.
Annealing/extension (+ plate read)	60°C (= primer Tm)	30 sec		
Extension + plate read (optional)	72°C	30 sec		
High Resolution Melting Analysis				
Heteroduplex formation	95°C	30 sec	1	
	60°C	1 min	1	
High resolution melting + plate read	65–95°C (in 0.2°C increments)	10 sec/step	1	A narrower melting range (amplicon Tm +/- 5°C) can reduce total run times.

6. Load PCR tubes or plate into the real-time PCR instrument and start the PCR/HRM run.

7. Perform PCR and HRM data analysis according to the instrument and software guidelines.

Note:

- A companion precision melt calibration kit (Catalog #184-5020) must be used to calibrate the CFX instrument prior to analysis with the Precision Melt Analysis™ software.

Related Products

- Precision Melt Analysis™ software (Catalog #184-5025)
- Melt calibration kit (Catalog #184-5020)
- Precision melt demo kit (Catalog #172-5116)

To learn more about Bio-Rad's complete solution for HRM, visit: **www.bio-rad.com/hrm.**

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