



# ORA™ aPCR HRM Mix, 2X

CAT.#	SIZE	COMPONENTS	COMPONENT COMPOSITION
QPD0301	200 r of 20 μl	2 x 1 ml - ORA™ qPCR HRM Mix, 2X	Hot Start qPCR components: dNTPs at 0.25 mM, optimized buffer,
		2 x 1 ml - PCR Water	proprietary saturating intercalating dye
QPD0305	1000 r of 20 μl	10 x 1 ml - ORA™ qPCR HRM Mix, 2X	Hot Start qPCR components: dNTPs at 0.25 mM, optimized buffer,
		10 x 1 ml - PCR Water	proprietary saturating intercalating dye
Storage	In the dark at -20°C.		

#### **APPLICATIONS**

High Resolution Melting analysis (HRM):

- Detection of sequence variations
- SNP genotyping
- Methylation analysis
- Mutation scanning

## PRODUCT DETAILS

High Resolution Melting analysis (HRM) is a fast and simple technique for identification of DNA sequence variations. It allows identifying single nucleotide differences by detecting minor changes in qPCR melting curves.

highQu ORA<sup>™</sup> HRM qPCR Mix includes a proprietary intercalating saturating dye showing no inhibition for PCR. The dye has the same affinity for both AT or GC rich sequences what leads to highest accuracy in genotyping.

The hot-start function in the mix is based on the small molecular inhibitor technology and allows achieving highest sensitivity and specificity under both standard and fast qPCR cycling conditions. The mix provides excellent performance on both AT and GC rich templates and reliable results with minimum or no optimization.

#### **BENEFITS**

- Time and costs saving analysis of sequence variations
- Universal standard or fast cycling, GC or AT rich templates
- · Highest sensitivity, no optimization required
- Supplied with PCR Water for maximum convenience

### **COMPATIBILE INSTRUMENTS**

Life	7500, 7500 FAST, 7900, 7900HT FAST, 7900HT, Viia™7,
Technologies:	QuantStudio™ 12K Flex
BioRad:	CFX96™, CFX384™
Eppendorf:	Mastercycler® ep realplex Mastercycler® realplex 2S
Illumina:	Eco
OIAGEN:	Rotor-Gene®Q, Rotor-Gene® 6000, Rotor-Gene®
QIAGLIN.	3000
Roche Applied	LightCycler®480, LightCycler®96, LightCycler®Nano
Science:	

# PROTOCOL

- Use special primer selection programs for good planning.
- Work with amplicons in a range of 80-200, max 400 bp.
- Take typical measures to prevent PCR cross over contamination, keep your bench clean, wear gloves, use sterile tubes and filter pipet tips.
- Run reactions in triplets; include a no-template control and positive control in parallel.
- Thaw and keep reagents on ice. Mix well before use.
- Do not perform annealing/extension for more than 30 seconds and do not use lower than 60 °C temperature for this step.

✓ Prepare a 20 µl reaction:

Reverse Primer	100 - 400 nM final c.	
Forward Primer	100 - 400 nM final c.	
cDNA Template or	<100ng <i>or</i>	
gDNA Template	<1 µg	
PCR Water	to 10 μl	
ORA™ HRM Mix, 2X	10 µl	

- ✓ Mix gently, avoid bubbles.
- ✓ Place into the instrument (SYBR® Green or FAM channel), set like:
  Initial denaturation 1 cycle: 95°C 2 min for cDNA, or
  1 cycle: 95°C 3 min for gDNA

  Denaturation 40 cycles: 95°C 5 sec

  Annealing/extension 40 cycles: 60-65°C 20-30 sec

Follow instrument instructions for melting curve analysis.

IN VITRO RESEARCH USE ONLY