2X HRM-OLC Mix Manual

Cat No: BS-AMP-106-500
BS-AMP-106-1000
BS-AMP-106-5000

Manual Ver.: 1711091110EAG

Table 1. Product Content and Storage Conditions - Shelf Life: 1 Year					
Component	**Ingredient**	**Piece**			**Storage**
		500 Test	**1000 Test**	**5000 Test**	
2X HRM-OLC Mix	2x qPCR mix and HRM Dye mixture	1x2500 µL	1x5000 µL	5x5000 µL	-20 °C

User Supplied Equipment and Consumables

1) Real Time PCR device (1 piece): FAM/SybrGreen Channel, Ramp Rate ≥3°C/s. **2)** 1-10 µL Micropipette (2 piece): Adjustable volume. **3)** 100 µL PCR tube passive cold block (1 piece). **4)** 2,0 mL tube passive cold block (1 piece). **5)** 1-10 µL Micropipette tip: DNaz-Free. **6)** qPCR Tube and lid/PCR Plate: Real time PCR compatible, DNaz-Free. **7)** Quick Spin Centrifuge (1 piece): Min. 3000 rpm. **8)** PCR Installation UV Cabinet (min. 1 piece): Working floor stainless steel; UV protection min. % 99,9.

Principle: High-resolution melting curve (HRM) analysis after Real-Time polymerase chain reaction. Double strand of amplified DNA sequences separated when the temperature increases. This separation provides a very specific graph according to length and content of amplicons. Using high-precision intercalation dyes, the reduction in fluorescence level due to separation of DNA double strand is determined by instant readings of qPCR, thus different sequences can be discriminated. DNA Polymerase in the mix possesses the 5'->3' DNA polymerase activity, 3'->5' (proofreading) exonuclease activity and temperature-depended strand-displacement activity and generates blunt ends in the amplification products.

Usage Purpose: Identification of sequence differences present in common loci in different organisms by amplification of target gene regions in nucleic acid isolates and high resolution melting curve (HRM) analysis.

Warnings: 1) *Bio-Speedy® 2X HRM-OLC Mix* is used for general laboratory applications. It is not recommended for any clinical purpose / medical diagnosis. It is the initiative of the user that these kits are in accordance with the analyzes to be used. 2) The product can be stored in small volumes for risk of loss of activation and contamination. It should be stored in dark place against loss of activity. In order to prevent contamination, it should be kept away from any DNA, RNA, and especially amplified nucleic acid source. 3) The content of the product should not be mixed with reagents of the same name but of different lot numbers or produced by different producers. 4) The shelf life of the product is 1 year. Products that have expired should not be used. 5) The product should be kept on the cold block, and the qPCR setup should be performed on the cold block if possible. It should be mixed with gentle shaking before use. 6) The area where the nucleic acid isolation is performed and the area where the qPCR installation is made should be different. Similarly, the area where the qPCR mixes are distributed to the tubes and the area where the template DNAs are distributed to the tubes should be in separate chambers; if this is not possible it should be ensured that they are in different benches. In addition, the micropipettes used to pipet the template DNA and the qPCR mixes must be separate sets. 7) Template DNA and positive control tubes should be kept closed except for reaction preparation. 8) qPCR Setup UV Cabinet should be exposed to UV for at least 15 minutes at the beginning and end of each working day. The surfaces of the chambers, benches and devices where the analysis is carried out should be regularly cleaned with 10% NaClO. 9) Clean and new gloves should be used during operation. 10) Completed qPCR reaction tubes should be disposed before they are opened in the laboratory.

APPLICATION PROTOCOL

Table 2. qPCR Setup: 1) Warnings must be read before installation. 2) The amount of components contained in the table is for 1 reaction. When requested, PCR-Mix (2X HRM Mix + Primers + Molecular Grade Water) can be prepared and distributed to PCR tubes at the same time as the total number of samples and control reactions. 3) When Real-Time PCR is programmed, Melting Curve Analysis should be set between 65 ° C and 95 ° C and Ramp Rate = 0.1 ° C / s (continuous reading).

Component	Setup Area	Adding Order	Target qPCR (For 1 reaction)	Neg. control qPCR (For 1 reaction)	Pos. control qPCR (For 1 reaction)
2X HRM-OLC Mix	qPCR mix setup area	1	5 µL	5 µL	5 µL
Primer-Forward (10 pmol/µL)		2	0,5 µL	0,5 µL	0,5 µL
Primer-Reverse (10 pmol/µL)		3	0,5 µL	0,5 µL	0,5 µL
Molecular Grade Water		4	2 µL	4 µL	2 µL
DNA (Sample)	Target DNA adding area	5	2 µL	-	-
PC (Pos. cont.)-Target		6	-	-	2 µL
TOTAL REACTION VOLUME =			10 µL	10 µL	10 µL

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