Microzone Product Datasheet

Product code: 2MMER-RTU-10 Lot Number:

MegaMix Emerald 2X RT-qPCR One-Step Mastermix, with UNG Separate ROX

P. Code	Size in 200 Rxn	Size in 1000 Rxn	Component	Description	Lot Number	Expiry
2MMEU-1	2 x 1 mL	10 x 1 mL	2X MegaMix Emerald qPCR Mastermix with UNG	Hot Start Taq, 0.2 mM dUTPs, 3 mM $MgCl_2$ thermolabile UNG and intercalating dye in optimised buffer (final concentrations).		
2EM-1	1 x 200 µL	5 x 200 µL	20X RT/RI Enzyme Mix	Concentrated combination of RT and RNase inhibitor.		
5JWA-1	2 x 1 mL	10 x 1 mL	Just Water (Molecular grade water)	Aliquoted, quality controlled, nuclease free, molecular grade water.		
ROX-0.1	1 x 100 µL	5 x 100 µL	25 µM ROX Reference Dye	Passive ROX reference dye		

Applications

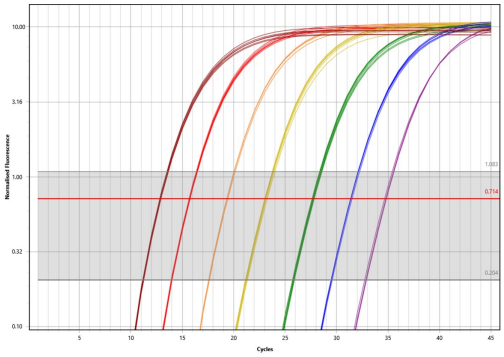
- Quantification of any RNA template (mRNA, total RNA, viral RNA), low copy number samples.
- Gene expression analysis.
- Pathogen detection.
- RT-qPCR assays using fluorescence of intercalating dye.
- High resolution melting.

Product Description

Containing all the components needed to perform RT-qPCR swiftly and reliably. The kit consists of 2X MME Mastermix, containing chemically modified Hot Start Taq DNA polymerase, intercalating dye, dUTP and UNG in enhancing buffer; 20X RTase/RI enzyme mix, optimised for amplifying low copy RNA targets; and Just Water, our molecular biology grade water. The third generation intercalating fluorescent dye binds to double stranded DNA, making MegaMix Emerald the perfect choice for qPCR, Melt Curve Analysis and High Resolution Melting (HRM). The Hot Start Taq polymerase is chemically inactivated until heating to 95°C, providing excellent sensitivity and specificity; eliminating the formation of non-specific amplification and primer-dimers. The presence of UNG and dUTP's eliminates carryover contamination, the thermolabile UNG is active at room temperature before being completely and irreversibly inactivated when heated to 95°C, this means your PCR product is suitable for downstream processing.

Key Features

- Specificity—Hot Start Taq DNA Polymerase in optimized buffer eliminates non-specific amplification and the formation of primer dimers.
- Sensitivity—Single copy detection.
- Eliminates carry over contamination—incorporation of thermolabile UNG and dUTP prevent amplicon contamination from previous runs without degrading generated amplicons.
- Versatile— compatible with standard and fast cycling conditions, GC/AT rich templates.
- Inhibitor Resistance—suitable for direct to PCR with products such as microLYSIS-RNA.
- Third generation intercalating dye— microGREEN dye does not inhibit PCR, even at high concentrations.



MegaMix Emerald demonstrates excellent reproducibility and accuracy across a wide dynamic range. RT-qPCR amplification of GAPDH gene from seven 10-fold dilutions of total avian RNA, six replicates at each concentration. Mic qPCR Cycler (BMS, Upper Coomera - Australia).

Protocol

This products is to be used as follows.

Thaw all reagents completely and mix well before use.

Prepare a master mix as described in the table below. This reaction can be scaled according to the quantity of reactions required.

Mix gently, avoiding bubbles, centrifuge if necessary.

Include a no template control and positive control as required.

Incubate the reactions at room temperature for 2-5 minutes prior to cycling to allow the UNG to eliminate carryover contamination.

Components	Volume
2X MegaMix Emerald qPCR Mastermix with UNG	10 µL
20X RT/RI Enzyme Mix	1 µL
25 µM ROX Reference Dye (Optional)	Low 0.04 µL / High 0.4 µL
Primer mix	x µL
Template	y µL
Just Water (Molecular grade water)	make up to 20 µL

The ROX concentration required will depend on the qPCR instrument in use.

Thermocycling

Transfer the reactions to the thermal cycler and set as follows:

Annealing temperature (60°C) may require optimisation depending on the specific primers in use.

The run time can be shortened by optimising the steps of the thermocycling profile.

Cycles	Temperature	Time
1	50°C	10 mins
1	95°C	2 min
40	95°C	3 sec
	60°C	20 sec

The included dye, microGREEN, has an absorption wavelength of 487 nm and a excitation wavelength of 511 nm, therefore acquisition can be performed in the FAM/SYBR channel of any compatible thermocycler.

Note: Low ROX instruments require a ROX final concentration of 50 nM and high ROX instruments require a ROX final concentration of 500 nM.

For research use only

Product Handling

Storage

To ensure the quality of the product until the expiry date keep at the recommended storage temperature and limit exposure to light.

Contamination Control

To prevent erroneous results ensure work environment is free of contamination by cleaning your workstation and equipment daily with a DNA decontaminant, wear gloves, use sterile tubes and filter pipette tips.

Simple | Effective | Efficient

Version 2.01

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