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



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

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 <sub>2</sub> 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.		

# **Applications**

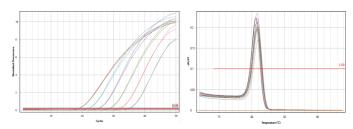
- Quantification of any RNA template (mRNA, total RNA, viral RNA), low copy number samples.
- Gene expression analysis.
- Pathogen detection.
- RT-qPCR assays using fluorescence of intercalating dye.
- High resolution melting.

## **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 optimised; 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.
- Reproducibility and convenience—ready to use 2X format.
- Inhibitor Resistance—suitable for direct to PCR with products such as microLYSIS-RNA.
- Third generation intercalating dye— no inhibition of PCR, even at high concentrations, excellent for High Resolution Melting.



MegaMix Emerald 2X One-step RT-qPCR with UNG shows excellent linearity and reproducibility with 6X 1 in 10 dilution series of a high concentration samples. Demonstrating excellent specificity with reproducible melt curves. Placental Human RNA, B2M, BMS MIC.

## 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

Components	Volume
2X MegaMix Emerald qPCR Mastermix with UNG	10 μΙ
20X RT/RI Enzyme Mix	1 μΙ
Primer mix	×μl
Template	уμΙ
Just Water (Molecular grade water)	make up to 20 μl

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

## Thermocycling

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

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

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.

The included dye 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 thermalcycler.

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 with a DNA decontaminant daily, wear gloves, use sterile tubes and filter pipet tips.

Simple | Effective | Efficient