



Universal Mastermix

Cat. No	Amount
000054	100 reactions

For research use only!

Description

The Universal Mastermix contains all the required reagents (except the primers and the DNA or RNA template) for the performance of LAMP analysis of DNA or RNA samples. The 2X Universal Mastermix is optimized to be used with the HNB dye, which is included in the kit, but it can also be used in combination with other colorimetric or fluorescent dyes, e.g. SYBR Green I or II. The Bst and Reverse transcriptase enzyme that are contained in the master mix can produce robust LAMP amplification in a very short running time. They can provide high sensitivity and specificity, allowing the specific amplification of DNA and RNA genomes and fragments, from low copy numbers.

The HNB dye which is included in the kit allows LAMP colorimetric analysis of the samples. Using this dye a positive sample can be discriminated from a negative one by the naked eye or automatically in real-time when it is used with the PEBBLE-R qCLAMP platform which is optimized to be used with. It is very easy to handle, and the results are easily discriminated.

Content

The Universal Mastermix contains the enzyme mix with the Bst polymerase and the Reverse Transcriptase, dNTPs, buffers, nuclease-free water, the HNB dye, and mineral oil.

Storage

All components should be stored at -20°C and avoid multiple freeze-thaw cycles, to guarantee maximal shelf life. After opening, the mineral oil can be stored at room temperature.

Shelf life

24 months

Shipping conditions

Shipped on ice bags or dry ice in liquid form.

Pack components

Component	000054
2X Universal Master mix	1.4 mL
HNB dye	0.12 mL
Mineral oil	1.8 mL

Activation of runs

In case you use the Universal Mastermix with the PEBBLE-R qCLAMP Platform device, before the usage of the kit, the PEEBLE runs should be activated. Use a smart device (smartphone or tablet) with the PEEBLE RUO application to scan the QR code that is located on the package of the product. When the QR code is successfully scanned the Universal Mastermix is ready to be used.



Standard protocol

The following protocol serves as a general guideline for the preparation of the master mix of all components except the template. Optimal reaction conditions (e.g. temperature, concentration of primers, and



concentration of DNA or RNA template) may vary depending on the target. The optimal reaction volume for a single reaction is 25 μ L.

1. Mix well and briefly centrifuge the 2X Universal Mastermix and the HNB dye after thawing. On ice prepare a mixture for the desired number of LAMP reactions following the volumes:

Component	Volumes per 1 reaction
2X Universal Mastermix	12.5 μ L
HNB dye	1 μ L
LAMP Primer mix 10X *	2.5 μ L
Template DNA/RNA	>10 copies
Nuclease-free water	Up to 25 μ L
Total volume	25 μL

*It is recommended for easy handling of the multiple primers used in the LAMP assay to prepare a concentrated Primer mix 10X by combining all the required primers.

2. Mix well the reaction. The color of the mix should be purple/deep blue.
3. Add carefully 15 μ L of mineral oil at the side of the vial and wait 30 sec until it forms a layer over the qcLAMP reaction. Make sure the oil is not mixed with the reaction mixture.
4. Incubate at 65°C for 30 minutes. The temperature and the time can be adjusted as necessary depending on the sequence of the primers and the concentration of the template.

Addition of template

The optimal amount of starting material may vary depending on the quality, stability, and the complexity of it. Both DNA and RNA templates can be used with the Universal Mastermix. It is recommended to use 10 copies or more of DNA or RNA templates.