Data Sheet (28.03.2024)

Universal Mastermix

2X Universal Mastermix for real-time quantitative colorimetric loop-meditated isothermal amplification (qcLAMP)

Cat.-No.	Amount
000054	1.4 ml (2X conc., 100 rxs x 25 µl)

For research use only!

Content

2X concentrated Universal Mastermix containing the enzyme mix with the *Bst* polymerase and the Reverse Transcriptase, 10X buffer, 100 mM Mg^{2+}, 10 mM dNTPs, nuclease-free water, 4 mM HNB dye, and mineral oil.

A QR code for 100 tests with the PEBBLE-R is included free of charge.

Pack components

Component	Final volume	Component Cat.-No.	Cap colour
Universal Mastermix (2X)	1.4 ml	BR054	Yellow
HNB dye (4 mM)	0.48 ml	BR056	Violet
Mineral oil	1.8 ml	BR055	Blue

Store at – 20 °C, avoid frequent thawing and freezing. After opening, the mineral oil can be stored at room temperature.

Shelf Life

24 months

Shipping conditions

Shipped on gel packs in liquid form

Description

The Universal Mastermix is a 2X concentrated *ready-to-use* mastermix which contains all reagents (except the DNA or RNA template and the primers) required for the performance of real-time quantitative colorimetric loop-meditated isothermal amplification (qcLAMP) of DNA or RNA samples. The 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. phenol red, SYBR Green I or II.

The *Bst* and Reverse transcriptase enzyme that are contained in the mastermix 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.

Standard Protocol

The following protocol serves as a general guideline for the preparation of the mastermix 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 µL.

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

Recommended assay x 25 µl

Component	Volume per 1 reaction
Universal Mastermix (2X)	12.5 µl
HNB dye (4 mM)	1 µl
LAMP Primer mix (10X)*	2.5 µl
DNA/RNA template	>10 copies
Nuclease-free water	Up to 25 µl

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

2. Mix well the reaction. The color of the mix should be purple/deep blue.

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3. Add carefully 15 µL 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.

Advantages of Using metabion Universal Mastermix in Molecular Biology Applications

1. **Compatibility with Various Sample Types**
   The Universal Mastermix does not use a pH-based dye such as phenol red, making it compatible with crude samples of varying pH levels, such as blood, urine, saliva, vaginal swabs, and nasopharyngeal swabs. This ensures high tolerance to impurities.
2. **Increased Stability**
   The absence of pH-based dyes and low buffering conditions enhances the stability of the Mastermix. Traditional mixes with pH-based dyes are prone to oxidation and pH changes after multiple freeze-thaw cycles. In contrast, Universal Mastermix maintains stability even after several freeze-thaw cycles.
3. **Fast Amplification Rate**
   The enzyme used in the Mastermix allows for rapid amplification, with target amplification at approx. 9-15 minutes. This increases both the efficiency and specificity of the amplification process.
4. **Thermostable *Bst* Polymerase**
   The *Bst* Polymerase in the Mastermix is highly thermostable, providing sensitive reverse transcriptase activity, which is crucial for accurate and efficient amplification.
5. **Low Limit of Detection**
   The Universal Mastermix boasts a low limit of detection, capable of detecting as low as 1-10 copies per µL. This makes it highly sensitive for various applications.
6. **Effective Multiplex Detection**
   The Mastermix performs well in multiplex detection scenarios, accommodating multiple primer sets simultaneously without compromising performance.
7. **Versatile Detection Method**
   Without the inclusion of a colorimetric dye within the Mastermix, it is thus suitable for fluorescent detection using dyes such as SYBR Green (Cat. No. **mi-E8021S/M/L**). This versatility allows for a broader range of detection methods.

BIOPIX-T

Manufacturer: BIOPIX DNA TECHNOLOGY S.A.
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