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MAST ISOPLEX® *DNA Lyo Plus*

DNA/LYO2/NCE 100 tests.

Intended Purpose: An optimised isothermal amplification kit designed to simplify the preparation of Loop-mediated isothermal amplification (LAMP) reactions. Intended DNA targets from samples of interest can be amplified by incorporation of in-house defined primer sets. The kit contains two reconstitution buffers providing different buffering conditions to enable flexible optimisation of the LAMP assay.

NOT FOR CLINICAL DIAGNOSTIC USE.
FOR INVESTIGATIONAL USE ONLY.

Contents

1. Ten tubes of lyophilized MAST ISOPLEX® *DNA Lyo Plus* pellets (PEL2). Each lyophilised pellet is sufficient for 10 assay reactions. Red cap tubes.
2. One tube of lyophilized Positive Control Primers (PCP2). Blue cap tube.
3. One tube of lyophilized Positive Control DNA (PCDNA2). (10pg/µl when reconstituted). Green cap tube.
4. One tube with 1.5ml of 0.5M Tris reconstitution buffer (RB). Yellow cap tube.
5. One tube with 1.5ml of 1M CHES CAPSO reconstitution buffer (RB2). Orange cap tube.
6. One tube with 1.5ml of molecular grade water (WTR). Black cap tube.

Storage and Shelf life

1. Store the unopened kit at 2°C to 30°C away from direct sunlight until the expiry date shown on the pack label.
2. Once reconstituted, MAST ISOPLEX® *DNA Lyo Plus* pellets, Positive Control Primers and Positive Control DNA should be aliquoted and stored at minus 20°C. Store all other components at 2°C to 30°C away from direct sunlight.
3. Reconstituted MAST ISOPLEX® *DNA Lyo Plus* pellets must be used within 8 weeks after first reconstitution of the pellet.
4. If MAST ISOPLEX® *DNA Lyo Plus* pellet mix is unused in assays on the day of reconstitution they can be stored at minus 20°C until required. Store in small aliquots to prevent multiple freeze-thaw cycles. Pellet activity is stable at this temperature for at least 8 weeks.
5. Reconstituted MAST ISOPLEX® *DNA Lyo Plus* pellets should not be freeze thawed more than 5 times.
6. Reagents removed from kits may be kept at 2°C to 30°C during experimental set up.
7. Return all reagents to the appropriate storage conditions immediately after use.
8. Positive control DNA (PCDNA2) can be stored at 2°C to 8°C if needed on the day of resuspension. For long term storage after reconstitution, store in small aliquots at minus 20°C to prevent multiple freeze-thaw cycles.

Warnings and Precautions

Precautions should be taken to prevent contamination of reagents in the MAST ISOPLEX® *DNA Lyo Plus* kit and samples. The kit is designed to be used in conjunction with in-house defined primer sets and by trained laboratory personnel only.
Reaction tubes should be kept closed at all times following addition of reagents and discarded without opening following use, according to local health and safety guidelines. To avoid any contamination with the amplified product, never open a vial after amplification.
Do not vortex reaction tubes. Ensure all reaction tubes are not scratched or cracked prior to use.

Materials required but not provided

1. DNA sample and primer sets to be provided by the user.
2. DNA from specimen sample should be obtained according to standard laboratory procedures.
3. Standard DNase free supplies such as assay reaction tubes, pipettes and pipette tips.
4. An instrument capable of performing isothermal incubation of reaction tubes at the desired temperature such as the Applied Biosystems (ABI) 7500 FAST REAL-TIME PCR system, the ESEQuant TS system or equivalent in-house thermocycler. The equipment should have a fluorescent reader with FAM detection channel for recognition of amplification products.

Procedure

Assay Reagent reconstitution

1. Reconstitute the MAST ISOPLEX® *DNA Lyo Plus* pellet (PEL2) using either RB or RB2.
   Use of RB (Yellow tube cap)
   a. Add 20µl of reconstitution buffer (RB) to resuspend the pellet.
   b. To this, add 66µl of molecular grade water (WTR).
   c. Mix contents by gently pipetting the reagent up/down.
   Use of RB2 (Orange tube cap)
   a. Add 25 µl of reconstitution buffer (RB2) to resuspend the pellet.
   To this, add 61µl of molecular grade water (WTR).
   c. Mix contents by gently pipetting the reagent up/down.

2. Reconstitute the Positive Control DNA (PCDNA2) as follows:
   a. Spin tube briefly in a microcentrifuge to ensure DNA is at the bottom of the tube.
   b. Add 50µl of molecular grade water (WTR) and leave to dissolve for 5 minutes.
   c. Mix gently by pipetting up and down several times.

3. Reconstitution of Positive Control Primers (PCP2) as follows:
   a. Spin tube briefly in a microcentrifuge to ensure primers are at the bottom of the tube.

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b. Add 20µl of molecular grade water (WTR) and leave to dissolve for 5 minutes.
c. Mix gently by pipetting up and down several times.

Assay set-up

Each reconstituted MAST ISOPLEX® *DNA Lyo Plus* pellet can be used for ten assay reactions (10µl per assay).
For each assay:

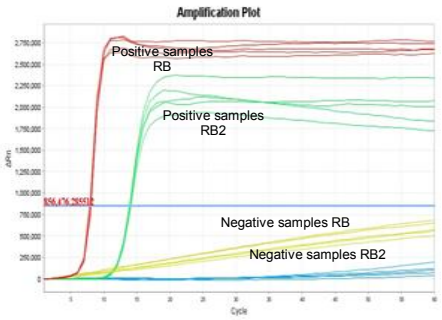
1. To a sterile DNase/RNase free reaction tube add 8.6µl reconstituted MAST ISOPLEX® *DNA Lyo Plus,* and 0.4µl in-house primer mix and 1µl DNA sample.
2. Gently pipette (do not vortex) to mix.
3. For a negative control, prepare a no-template control (NTC) by replacing 1µl of sample DNA with 1µl of molecular grade water (WTR) per assay.
4. For a positive control, replace sample DNA and in-house primers with 1µl of kit Positive Control DNA (PCDNA2) and 0.4µl positive control primers (PCP2) per assay.
5. Place assay reaction tubes in the chosen incubation equipment and start the reaction. LAMP assays run using these reagents should be performed at 63°C. Assays can work within a temperature range of 60°C to 65°C with mixed efficiencies.
6. If an assay is positive, fluorochrome detection will be detected within 40 minutes if using RB. Amplification of the positive control using RB is detectable within 20 minutes. If using RB2, fluorochrome detection will be detected within 40 minutes. Amplification of the positive control using RB2 is detectable within 30 minutes.

Note:

- The intercalating fluorochrome dye present in the MAST ISOPLEX® *DNA Lyo Plus* pellets can be read by a FAM filter setting.
- Recommended primer concentration per 10µl assay for in-house primer sets are:
  F3 and B3 – 2.5pmol
  FIP and BIP – 20pmol
  LoopF and LoopB – 10pmol
- If DNA sample volume needs to be greater than 1µl/reaction, the pellet can be resuspended in an appropriate quantity of reconstitution buffer and water to bring the concentration of the assay to optimum guidelines. Using a smaller volume of water (see Assay Reagent reconstitution section, point 1b) is the most effective way of doing this.

Interpretation of results

Thermocycler results: A positive result is indicated by presence of an amplification curve and a negative result is indicated by fluorescence without amplification within the reaction time as shown for
Positive sample: MAST ISOPLEX® *DNA Lyo Plus* Positive DNA
Negative sample: no template control
Example: ABI 7500 FAST REAL-TIME PCR system



Quality control

It is recommended that quality control on MAST ISOPLEX® *DNA Lyo Plus* pellets is performed using the Positive Control DNA (PCDNA2), the Positive Control Primer (PCP2), molecular grade water (WTR) and one of the pellet reconstitution buffers (RB or RB2) supplied in the kit per test run. These tests will ensure the reagents perform as specified and no contamination of kit reagents has occurred. If control reactions give incorrect results check for signs of deterioration or contamination of kit reagents before use.
Do not use kit reagents if deterioration or contamination is suspected.

Limitations of use

These products are for use in the amplification of DNA sample using in-house DNA extraction methods and in-house defined primer sets. Quality Control will determine if kit reagents and controls are functional and free of contamination but cannot determine potential issues the user may experience with in-house designed LAMP primer sets and samples for DNA extraction. Primer design and sample extraction is the sole responsibility of the end user. Results obtained with this kit must be considered alongside other clinically relevant data when diagnosing an infection.

References

Notomi T et al. Nucleic Acids Research (2000) 28 12, 63