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mast Isoplex® RNA

CE

REF 67rnamp1	48 Tests
REF 67rnamp2	96 Tests
REF 67rnamp3	192 Tests

Intended Purpose

Mast Isoplex® *RNA Amp*: For the isothermal amplification of an intended RNA target from a sample by incorporation of in-house defined primer sets. Mast Isoplex® *RNA Amp* reagents can be used for IVD testing, if the primer sets selected by the user are validated in combination with Mast Isoplex® *RNA Amp* kit components.

Composition

	48 Tests	96 Tests	192 Tests
Reaction Mix (RM) 2x conc.; yellow cap vial	1x 1mL	2x 1mL	3x 1mL
Bst Polymerase Enzyme; (EM) white cap vial	1x 55µL	1x 110µL	1x 220µL
Reverse Transcriptase (rEM); transparent cap vial	1x 28µL	1x 55µL	1x 110µL
Fluorochrome Dye (FD); Green cap vial	1x 55µL	1x 110µL	1x 220µL
Detection Dye (DD); blue cap vial	1x 55µL	1x 110µL	1x 220µL
Positive Control Primer Set (PM); pink cap vial	1x 25µL	-	-
Positive Control (PC); red cap vial	1x 40µL	-	-
Distilled Water (DW); transparent cap vial	1x 1mL	1x 1mL	2x 1mL

Storage and Shelf life

Store the unopened kit at -20°C until the expiry date shown on the pack label. Reagents should be stored at -20°C once opened and used up within 8 weeks after first opening the vial. Freeze thawing cycles of kit reagents should be kept as low as possible. Do not freeze-thaw the Fluorochrome Dye and Detection Dye more than 4 times. Alternatively it is recommended to store the Fluorochrome and Detection Dye at 2-8 °C. The product shelf life is one year.

Warnings and Precautions

Mast Isoplex® *RNA Amp* kit should be used in conjunction with in-house defined primer sets and by qualified personnel only. Reaction tubes should be kept closed at all times following addition of reagents and should be discarded, without opening following use, as per local guidelines.

Materials required but not provided

- RNA sample and target primer sets to be provided by the user.
- Standard RNase free supplies such as reaction tubes, pipettes and pipette tips.
- An instrument capable of isothermal incubation of reaction tubes at the desired temperature such as ESE*Quant* TS or thermocycler. When using the Fluorochrome Dye (FD) the equipment should have a fluorescent reader with FAM setting (real time thermocycler). Visual detection can be conducted using Detection Dye (DD).
- The kit is compatible with Eiken turbidimeter instruments LA320C and LA200.

Specimen sample

RNA from sample should be obtained as per standard procedure.

Procedure

When conducting more than one reaction prepare a Master Mix containing enough mix for an extra reaction to compensate any imprecisions in the pipetting volumes.

Note: Keep reaction tubes on ice or in a cold block during addition of reaction components. Reagents removed from kits should be placed and thawed out on ice. Avoid repeated freeze/thawing cycles. Do not mix reagents vigorously in the reaction tube. Do not vortex the enzyme mixes. Ensure reaction tubes are not scratched or cracked prior to use.

Per single reaction:

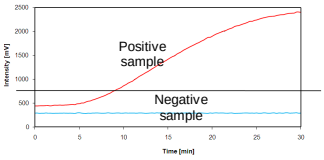
1. To a sterile DNase/RNase free reaction tube add 12.5µL of Reaction Mix (RM) and 1µL of Fluorochrome Dye (FD) or Detection Dye (DD).
2. Mix contents by gently pipetting the reagents up/down.
3. Add the required volume of a primer mix (primer mix volumes may vary between 1-2.5µL; for positive control reaction, add 2µL of Positive Control Primer set. (Optional: it may be necessary to boil the Master Mix for 5 minutes at 95°C at this stage to melt any primer multimers. The primers supplied in the kit for the Positive Control do not need this optional step. After heating place the Master Mix on ice for 5 minutes.)
4. Add 1µL Bst Polymerase (EM) and 0.5µL Reverse Transcriptase (rEM). **Caution:** do not vortex after adding the enzymes.
5. Add Distilled Water (DW) to give a total volume of 25µL following addition of RNA sample. Mix the reagents by gently pipetting up/down (do not vortex).
6. Add 2.5 to 5µL of sample RNA. For positive control reaction, add 5µL of Positive Control (PC). Mix by gently tapping the reaction tube or pipetting up and down (do not vortex). Spin reactions down.
7. Place tubes in appropriate incubation equipment and start the reactions. Reaction temperature depends on the primer set and may range from 60 to 65°C. Reaction times range from 30 to 60 minutes.

Note: if the Fluorochrome Dye is used, reaction times are shorter than for the visible detection reagent.

Interpretation of results

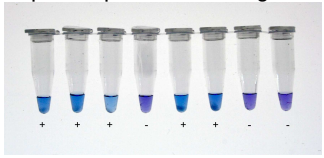
Fluorochrome Dye (FD) results: A positive result is indicated by presence of an amplification curve and a negative result is indicated by no fluorescence being detected.

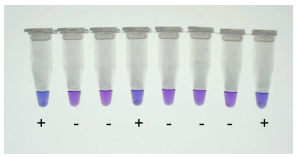
Example: ESE*Quant* TS results
Red: indicates positive sample
Blue: indicates negative sample



Reproduced from ESEQuant TS Tube Scanner Software manual

Detection Dye (DD) results: Depending on the RNA source the colour may vary in intensity and staining. A positive result will be observed as a bluish coloured reaction and a negative result as a bluish-purple coloured reaction. Note: The following pictures show examples of positive and negative results.

A. 

B. 

Limitations of use

These products are for use in the amplification of RNA sample using in-house defined primer sets or Mast Isoplex® primer sets.

Quality control

It is recommended that quality control is performed using the Positive Control (PC) supplied in the kit per test run. A negative control, using DNase/RNase free water during each test run is recommended. These tests will ensure the reagents are performing as specified and no contamination of kit reagents has occurred. Do not use the reagents if the control reactions are incorrect. Check for signs of deterioration or contamination of kit reagents before use.

References

[1]Notomi T *et al*. Nucleic Acids Research (2000) **28** 12, 63