Nu-LAMP™ TB Kit

(Loop mediated isothermal amplification TB Test Kit based on Real Time PCR/Visual Detection)

LM-TB-20 : 20 rxns
LM-TB-50 : 50 rxns
LM-TB-100 : 100 rxns

IVD

Product Insert

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Introduction

Mycobacterium tuberculosis (MTB) is a major cause of tuberculosis. Tuberculosis is a disease with serious mortality worldwide. Identification of MTB for the diagnosis of tuberculosis is largely based on conventional approaches, which rely on acid-fast bacilli (AFB) staining and culture. AFB staining is comfortable and rapid, but lacks sensitivity. Culture method is sensitive and specific, but is slow and extremely time consuming. The molecular methods, especially polymerase chain reaction (PCR) technique are the most promising with high sensitivity and specificity, but require more technical expertise and expensive lab setup. The Nu-LAMP™ TB kit has increased specificity and sensitivity on par with MTB Nested PCR and is economical, rapid with minimal technical expertise and minimal lab setup.

Intended Use

The Nu-LAMP™ TB kit is used to detect Mycobacterium tuberculosis (MTB) complex (*Mycobacterium africanum, Mycobacterium bovis, Mycobacterium canettii, Mycobacterium microti, Mycobacterium tuberculosis*) in Body fluids (CSF, Pleural Fluid, Ascitic Fluid and Synovial fluid) Sputum, Pus, Menstrual fluid, Urine and Tissue. After nucleic acid extraction loop mediated isothermal amplification, is performed either on a Real Time PCR machine and data is collected in the form of real time curve or on heating block and end point detection is done under UV light at the end of reaction. . Combined with other methods of biological investigation (clinical picture and other laboratory markers), the results obtained with the Nu-LAMP™ TB Kit are used to detect MTB infection .

Product Description

Nu-LAMP™ TB kit is an *in-vitro* diagnostic loop mediated isothermal amplification test for the detection of Mycobacterium tuberculosis (MTB) complex (*Mycobacterium africanum, Mycobacterium bovis, Mycobacterium canettii, Mycobacterium microti, Mycobacterium tuberculosis*) in human clinical samples. The assay principle is based on LAMP (loop mediated isothermal amplification) technique where Bst polymerase is used which has dual activity of amplification and strand displacement. The improved sensitivity of the assay is due to large amount of amplicon generated by Bst polymerase and detection in Real time PCR.

The kit contains all the reagents necessary for performing Qualitative Nu-LAMP™ TB assay. The target region selected in the pathogen genome is *rpoB* region of MTB complex.

Recommended Work areas

Molecular Diagnostics work area includes:

a) Sample preparation area/room – for extraction of nucleic acids from clinical samples
b) LAMP setup area/room - for setting up LAMP reaction
c) LAMP area/room – for performing LAMP using Real time PCR/Thermocycler

As part of Good Laboratory Practices (GLP), it is recommended to have dedicated areas to avoid cross contamination.

General Precautions

Precautions while extracting Nucleic acid

Always wear proper attire (powder free gloves, facemask and Head cap) before starting the nucleic acid extraction procedure. During preparation of samples, compliance with good laboratory practices are essential to minimize the risk of cross-contamination between samples, and the inadvertent introduction of Deoxyribonucleases (DNases) into samples during and after the extraction procedure. Proper aseptic technique should always be used when working with DNA.

The Sample Preparation Area is dedicated to processing samples. All reagents used in the Sample Preparation Area should remain in this dedicated area at all times. Laboratory coats, pipettes, pipette tips and vortex mixer used in the Sample Preparation Area must remain in this area and not be moved to the LAMP setup/LAMP area. Discard the gloves before leaving this area. Do not bring amplified product into the Sample Preparation Area. Usage of filter tips is recommended while sample preparation and should be performed in a Biosafety cabinet.

Precautions while setting up a LAMP reaction

LAMP assay is sensitive and any accidental introduction of product from previous amplification reactions leads to incorrect results. Hence measures to reduce the risk of contamination in the laboratory should include physically separating the activities involved in performing LAMP and complying with good laboratory practices.

It is recommended to have proper cleaning procedures to minimize the risk of cross contamination and carry over contamination (e.g. DNA OUT™, RNase OUT™, 0.1% Sodium Hypochlorite, Fumigation etc.).

It is recommended that areas should be defined in LAMP setup room for preparation of mastermix and addition of templates. Laboratory coats and equipment used in the LAMP setup Area must remain in this area and should not be moved to the Sample Preparation Area.

Precautions for post LAMP setup or Equipment area/room

The instrument/s used for setting up LAMP reaction should be kept in a separate segregated area away from Sample preparation area and LAMP setup area.

Precautions after completion of LAMP assay

The reaction tubes or strips should be properly discarded after the completion of run to avoid carry over contamination.

Usage Limitations

1. The kit and all its components are for *in-vitro* diagnostics only.
2. The product is to be used by personnel specially trained in the *in-vitro* diagnostics procedures only.
3. Follow the product insert strictly for optimal results.
4. Do not use the kit beyond the expiry date mentioned on the kit box.
5. Follow the guidelines provided in product insert for sample collection, storage and transport.
6. For ideal performance, store the kit under recommended conditions only.

Safety Precautions

1. All patient specimens should be considered as potentially infectious and handled in a BSL2 biosafety hood with BSL3 practices.
2. Wear personal protective equipment, including gloves, head cap, face mask and lab coats when handling kit reagents/sample extraction. Wash hands thoroughly using detergents before and after performing the test.
3. Do not smoke, drink or eat in areas where kit reagents and/or human specimens are being used.
4. Dispose of unused kit reagents and human specimens as per regulatory guidelines.

Storage Conditions and Product Stability

1. All the kit reagents should be stored at -20 °C. Replace all the kit components immediately at -20 °C after usage.
2. Repeated thawing and freezing (more than 6 x) of all kit reagents should be avoided, as it reduces assay sensitivity. If needed, make aliquots of the kit reagents according to the volume used in the protocol prior to freezing.
3. Allow reagents to be thawed completely on Ice/4 °C prior to use.
4. Kit reagents are stable through the end of the expiration date indicated on the box when stored at -20 °C.

Symbols

Description of Symbol	Denotation
	*in-vitro* Diagnostic medical device
	Consult Instruction manual (Product Insert) for use
	Lot Number of the kit or components
	Catalogue number
 <N>	Contains sufficient for <N> reactions (Pack Size)
	Manufacturer
	Temperature limitation (Storage Condition)
	Use by MMM-YYYY (Expiry Date)
	Biological risk (handle carefully)
	Important Note

Kit Components

Cap Color	Contents	Description	20 Rxns (LM-TB-20)	50 Rxns (LM-TB-50)	100 Rxns (LM-TB-100)
Grey	RAS TB CBRB	Amplification Reaction Buffer	1 x 340 µL	1 x 850 µL	2 x 850 µL
Blue	RAS Bst Enzy	DNA Polymerase enzyme	1 x 30 µL	1 x 75 µL	2 x 75 µL
Lilac	RAS LTB PC	Positive Control	1 x 30 µL	1 x 60 µL	2 x 60 µL
Brown	RAS FDR	Fluorescent Detection Reagent	1 x 30 µL	1 x 75 µL	2 x 75 µL
White	MBGW	Molecular Biology Grade Water	1 x 0.5 mL	1 x 0.5 mL	2 x 0.5 mL

Materials required but not supplied

The materials which are required but not supplied are listed below:

1. DNA Extraction kit
2. Biosafety Cabinet
3. PCR Hood
4. Calibrated variable micropipettes
5. Sterile pipette filter tips (aerosol free)
6. Vortex mixer
7. Dry Bath
8. Benchtop centrifuge with rotor for 1.5 mL reaction tubes
9. Thermocycler/Real Time PCR machine
10. Strip Tubes and Caps (0.2 mL) or PCR Tubes (0.2 mL) or 96 well plate
11. Cooling block (96 x 0.2 mL tubes)
12. 1.5 mL centrifuge tubes
13. 1.5 mL centrifuge tube stand
14. Cooling block (2 mL tubes)

15. Sterile powder free gloves
16. Facemask
17. Head cap
18. Lab coats
19. UV Illuminator 254nm or Blue light fluorescence detector

Quality Systems

In accordance with ISO-certified Quality Management System (9001:2008 and 13485: 2003) of RAS Lifesciences, each lot of Nu-LAMP™ TB kit is tested against predetermined specifications to ensure consistent product quality.

Sample Type/Collection/Storage/Transport

Sample Type

Body fluids (CSF, Pleural Fluid, Ascitic Fluid and Synovial fluid) Sputum, Pus, Menstrual fluid, Urine and Tissue).

Sample Collection, Storage and Transport

Please follow the guidelines mentioned below for collection, storage and transport of different types of samples. The yield and quality of extracted DNA would vary based on the quality of sample received and if the following conditions are not followed.

Sample Type	Collection Requirement	Transport	Storage/ Processing
Body Fluids	1 mL of Body fluids in a sterile Container	In a leak proof box containing frozen cool packs (4 ^{0}C)	Store the sample at 4 ^{0}C /Sample to be processed for DNA extraction within 24 hours
Tissue	10-20 mg of tissue in a sterile container containing 1X PBS	In a leak proof box containing frozen cool packs (4 ^{0}C)	Store the sample at 4 ^{0}C /Sample to be processed for DNA extraction within 24 hours
Sputum, Pus, Menstrual fluid and Urine	1 mL of Sample in a sterile container	In a leak proof box containing frozen cool packs (4 ^{0}C)	Store the sample at 4 ^{0}C /Sample to be processed for DNA extraction within 24 hours

Assay Procedure

DNA Extraction

Nu-LAMP™ TB kit has been validated using RAS DNA Extraction Kit (Cat. No. R-DXT) Follow the manufacturer's instructions mentioned in the manual for DNA extraction. However the customer can validate their own DNA extraction systems.

ⓘ The analytical sensitivity and specificity of Nu-LAMP™ TB kit is validated using RAS DNA Extraction Kit.

LAMP Assay Protocol

1- Take out the kit components and place them on Ice/-20 °C mini cooler.
2- Thaw the components (Except RAS Bst Enzy) and spin down the contents, if necessary.
3- Use separate PCR reaction tubes/strips for each Sample/LTB PC/ Negative Control (MBGW).
4- Prepare the reaction mastermix and dispense the components per reaction as given in the table below.

Components	Volume in µL (25 µL Reaction)
RAS TB CBRB	17.0
RAS FDR	1.5
RAS Bst Enzy	1.5
RAS LTB PC/Test Sample/MBGW	5.0
Total	25.0

Note:** **1.** *For Instruments requiring passive reference dyes* ***(ABI 7500Dx, ABI Step one), 0.4 µL of ROX Low *has to be added to the reaction mix/sample so that the final reaction volume will be* ***25.4 µL.*** *Real Time PCR instruments (****Rotor-Gene™ 6000, Rotor-Gene™ Q 5plex****) which do not require passive reference dye, there is no need to add* ***ROX Low*** *and the final reaction volume will remain* ***25.0 µL***

2. *Difference in final reaction volume from* ***25 to 25.4 µl*** *does not make any difference in the sensitivity of the assay.*

5- Place the PCR plate/tubes/strips in thermocycler/ Real time PCR machine.
6- Incubate the tubes at 65 ^{0}C for 60 minutes for Thermocycler.
7- for Real time PCR

No of time points/ No of cycles	Temperature in ^{0}C	Time in minutes
60	65*	1min
*Plate read in SYBR green/FAM channel, Set Gain optimization also at 65^{0}C		

Switch off the Lid temperature if using thermocycler

8- After completion of the run, analyze the run.

Result and Interpretation

Data Analysis (when using Thermocycler)

Analyze the results by observing the color change in tube when kept under UV light at short range wavelength (254 nm).

Result and Interpretation (when using Thermocycler)

Observation	Interpretation	Conclusion
Fluorescent Green color observed in positive control/samples ; No color observed in negative control	MTB DNA Detected	Proceed for further Analysis
Fluorescent Green color observed in positive control; No color observed in samples/negative	MTB DNA Not detected	

Note: ***Results can be visually observed using UV illuminator at Short range wavelength (254 nm) or Blue light fluorescence detector***

Data Analysis (when using Real time PCR machine)

Analyze the data after completion of the run. Check the *Rn/Cycle* amplification plot and Δ*Rn/Cycle* amplification plot to observe the amplification signal

Result and Interpretation (when using Real time PCR machine)

The Amplification time (At) for unknown samples and PC would appear in the result column in SYBR green/FAM Channel. The negative control should not show any

value in the in SYBR green/FAM Channel. Analyze the results by observing the amplification time for samples, PC and NTC.

Observation	Interpretation	Conclusion
Amplification time observed in positive control and samples; No Amplification time observed in negative control	MTB DNA Detected	Proceed for further analysis
Amplification time observed in positive control; No Amplification time observed in negative control and samples	MTB DNA Not Detected	

Troubleshoot

Observation	Possible cause	Solution
No Amplification time observed in positive control/ samples/negative control	1- Improper reaction setup 2- Instrument was not calibrated 3- Degradation of kit reagents due to improper storage	1- Repeat the assay 2- Check for instrument calibration status 3- Repeat the assay with fresh aliquot of reagents

For any other technical query; please contact amplisure@raslifesciences.com

Assay Characteristics

Analytical Sensitivity

To determine the analytical sensitivity Nu-LAMP™ TB kit, plasmid DNA control and positive genomic DNA samples ranging from 15 copies/reaction to 1 copy/reaction were tested using the Nu-LAMP™ TB kit as described in the table below. The

different copy number templates were generated by serial dilution of the positive genomic DNA samples and plasmid DNA control. The testing was performed in triplicates for 5 consecutive days.

Sample Concentration (Copies)	Day 1 Result Triplicates	Day 2 Result Triplicates	Day 3 Result Triplicates	Day 4 Result Triplicates	Day 5 Result Triplicates
15	+ + +	+ + +	+ + +	+ + +	+ + +
10	+ + +	+ + +	+ + +	+ + +	+ + +
5	+ + +	+ + +	+ + +	+ + +	+ + +
3	+ + +	+ + +	+ + +	+ - -	- - -
1	- - -	- - -	- - -	- - -	- - -

The analytical sensitivity of Nu-LAMP™ TB kit is **5 copies per reaction.**

Analytical Specificity

The analytical specificity of the Nu-LAMP™ TB kit for MTB complex was assured by primer design and stringent PCR conditions. Blast search was done to avoid any homology on primers with other organisms.

MTB Complex Panel	Result
Mycobacterium tuberculosis	+
Mycobacterium microti	+
Mycobacterium africanum	+
Mycobacterium bovis	+
Mycobacterium canettii	+

The specificity of primers was checked for the following possible cross reactive pathogens utilizing International positive controls/ confirmed positive samples and human DNA.

Organism/Sample Tested	Result
Mycobacterium smegmatis	-
Mycobacterium fortuitum	-
Mycobacterium kansasii	-
Mycobacterium phlei	-

Mycobacterium vaccae	-
Mycobacterium wolinskyi	-
E. coli	-
Citrobacter sps	-
Nocardia sps	-
Cryptococcous sps	-
Salmonella sps	-
Vibrio Cholera	-
Listeria sps	-
Campylobacter sps	-
Staphylococcus aureus	-

Precision

The precision data of the Nu-LAMP™ TB kit has been generated with MTB positive clinical specimens.

Intra-assay variability

The precision data consists of the *Intra-assay variability* which is variability of multiple results of samples of the same copies within one experiment.

Inter-assay variability

The precision data consists of the *Inter-assay variability* which is variability of multiple results of samples of the same copies within different experiments.

Testing was performed with 3 replicates for 10 days.

Day of Testing	Sample 1 Test result (10 copies)	Sample 2 Test result (5 Copies)	Sample 3 Test result (2.5Copies)
1	+ + +	+ + +	+ + +
2	+ + +	+ + +	+ + +
3	+ + +	+ + +	+ + +
4	+ + +	+ + +	+ + +
5	+ + +	+ + +	+ + +
6	+ + +	+ + +	+ + +
7	+ + +	+ + +	+ + +
8	+ + +	+ + +	+ + +
9	+ + +	+ + +	+ - -
10	+ + +	+ + +	- - -

Abbreviations

Abbreviation	*Expansion*
MTB complex	Mycobacterium tuberculosis complex
LAMP	Loop Mediated Isothermal Amplification
DNA	Deoxy Ribonucleic Acid
IC	Internal Control
DNases	Deoxy Ribonucleases
PCR	Polymerase Chain Reaction
BSL2	Bio Safety Level 2
BSL3	Bio Safety Level 3
mL	Milli Liters
µL	Micro Liters
K_2EDTA	Potassium Ethylene Diamine Tetra Acetate
g	Relative Centrifugal Force
MBGW	Molecular Biology Grade Water
NTC	No Template Control
BLAST	Basic Local Alignment Search Tool
Sps	Species
Rxn	Reaction
FDR	Fluorescent Detection Reagent
At	Amplification time
CBRB	Complete Bst Reaction Buffer

References

1. Latchnik J *et al*. Rapid cycle PCR and fluorimetry for detection of mycobacteria. J Clin Microbiol 2002; 40(9): 3364-73.

2. Kirschner P *et al*.Diagnosis of mycobacterial infections by nucleic acid amplification: 18- month prospective study. J Clin Microbiol 1996; 34(2): 304-12.

Ordering Information

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