RAS DNA Extraction Kit

R-DXT-20 : 20 rxns
R-DXT-50 : 50 rxns

Product Insert

RAS Lifesciences Pvt. Ltd
Plot No. 13, 4-7-18/13/2., Raghavendra Nagar,
Nacharam, Hyderabad 500 076, India
Tel: +91-40-65261562,
www.raslifesciences.com

Index

Introduction

RAS DNA Extraction Kit provides a simple, nontoxic method for efficiently isolating high-molecular-weight DNA (Genomic DNA) from tissue and other body fluids. Depending on the starting material, the entire extraction takes only two to three hours to complete and does not require phenol or chloroform. DNA isolated with the RAS DNA Extraction Kit is free from contaminants and may be used directly for restriction digests, cloning, PCR amplification, and other DNA analysis techniques.

Intended Use

RAS DNA Extraction kit is used to extract TB DNA from Clinical samples by salt precipitation. The extracted TB DNA is used as sample for in-vitro diagnostic determination of TB by Loop Mediated Isothermal Amplification (LAMP) method.

Product Description

The DNA Extraction Kit is a modification of a procedure based on separating contaminating protein from DNA by salt precipitation. The procedure involves digestion of cellular proteins, subsequent removal of the proteins by salting out, precipitation of the DNA with Isopropanol and re-suspension in the buffer of choice.

Recommended Work areas

Molecular Diagnostics work area includes:

a) Sample preparation area/room – for extraction of nucleic acids from clinical samples
b) Pre-PCR area/room - for setting up PCR reaction
c) PCR area/room – for performing PCR using the thermocyclers/real time PCR

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, open one sample tube at a time and close it before opening another tube, follow it at every step of isolation. Compliance with good laboratory practices is essential to minimize the risk of cross-contamination between samples, and the inadvertent introduction of Nucleases into samples during and after the extraction procedure. Proper aseptic technique should always be used when working with Nucleic acid.

The Sample Preparation Area is dedicated to processing samples. All reagents used in the Sample Preparation Area should remain in this dedicated area. Sample Preparation Area must have dedicated laboratory coats, pipettes, pipette tips and cyclo-mixer and not to be moved to other areas. Discard the gloves before leaving this area. Do not bring amplified product into the Sample Preparation Area. Sample preparation should be performed in a Biosafety cabinet using aerosol free tips.

Usage Limitations

1. All the kit 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 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.

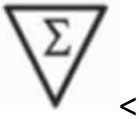
Storage Conditions and Product Stability

1. All the kit reagents should be stored at specified storage temperatures mentioned on respective reagent bottle/vial.. Replace all the kit components immediately at specified storage temperatures 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 reagent RAS PK 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 specified temperature/s.
5. Do not substitute or mix reagents from different kit lots or from other manufacturers.
6. Do not interchange reagent tube / bottle caps as this may lead to contamination and compromise test results.
7. Only use the protocol provided in this kit insert. Alterations to the protocol and deviations from the times and temperatures specified may lead to erroneous results.
8. On receipt of the kit, store the individual contents of the kit as detailed below

Component	**Storage Conditions**
RAS LB	2-8 ^{0}C
RAS PK	-20 ^{0}C
RAS SDS	-20 ^{0}C
RAS LYZ ENZY	-20 ^{0}C
RAS Poly A	-20 ^{0}C
RAS PPS	Room Temperature
RAS TEB	Room Temperature

Note: Keeping the kit reagents in frozen condition doesn't affect their stability and activity

Symbols

Description of Symbol	Denotation
	*in-vitro* Diagnostic medical device
	Consult Instruction manual (Product Insert) for use
	Lot Number of the kit or Kit contents
	Catalogue number of Kit
 <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 (coding)	Contents	Description	20 rxns (R-DXT-20)	50 rxns (R-DXT-50)
Natural	RAS LB	Lysis Buffer	1 x 50 mL	1 x 125 mL
Purple	RAS PK	Proteinase K solution	1 x 20 µL	1 x 50 µL
Orange	RAS SDS	Denaturing Solution	1 x 200 µL	1 x 0.50 mL
Blue	RAS LYZ Enzy	Lysozyme solution	1 x 380 µL	1 x 1.0 mL
Grey	RAS Poly A	DNA precipitator	1x 70 µL	1x154 µL
Natural	RAS PPS	Protein Precipitating Solution	1 x 3.5 mL	1 x 9.0 mL
Natural	RAS TEB	Tris EDTA Buffer	1 x 2.0 mL	1 x 5.0 mL

Materials not supplied

1. Ground glass conical homogenizer or other manual or mechanical tissue disruptor
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. 1.5 mL centrifuge tubes
10. 1.5 mL centrifuge tube stand
11. Sodium Citrate
12. 10N NaOH
13. N-Acetyl L-Cysteine
14. Isoproponol (Molecular Biology grade or equivalent)
15. Small box containing Ice
16. Nuclease free Water
17. Sterile Nitrile gloves
18. Facemask
19. Head cap
20. Lab coats
21. 70% Ethanol

Quality Systems

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

Sample Type/Collection/Storage/Transport

Sample Types

TB Culture, Blood- EDTA, Body fluids (CSF, Pleural Fluid, Acitic Fluid and Synovial fluid) Sputum, Pus, Menstrual fluid, Urine and Tissue.

Heparinized Blood must not be used as they inhibit the PCR reaction

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/ Blood – EDTA/TB Culture	1 ml of sample in a sterile container	In a leak proof box containing frozen cool packs (4^0C)	Store the sample at 4^0C /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 (4^0C)	Store the sample at 4^0C /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 (4^0C)	Store the sample at 4^0C /Sample to be processed for DNA extraction within 24 hours

Sample material should be transported in a leak proof, unbreakable transport container to avoid leakage of sample. The samples should be transported following the local and national instructions for the transport of pathogen material.

Procedure

Note: ***Always Prepare a fresh Lysis Mix before performing the DNA extraction. For a single extraction: the composition the composition of Lysis Mix is 280 µL of RAS LB and 2.8 µL of RAS Poly A.***

Protocol for DNA Extraction from Fluids (Urine, Ascetic Fluid, Pleural Fluid, CSF and BAL Fluid)

1. Take 1 mL of sample in the microcentrifuge tube spin 10,000 rpm for 1min.
2. Discard the supernatant. Leaving small amount of liquid (approx 100 µL)
3. Add 282.8 µL Lysis Mix to the sample.
4. Add 19 µL of RAS LYZ Enzy and incubate at 65 ^{0}C for 10 minutes.
5. After incubation, cool the homogenate and add 10 µL of RAS SDS and 1 µL of RAS PK.
6. Incubate for 1 hour at 65°C in heating block. (Depending upon lysis of sample, incubation time can be reduced)
7. After incubation, cool the homogenate by keeping the microcentrifuge tube in ice for 5 minutes.

8. Add 175 µL of RAS PPS to the homogenate and invert tube 10-20 times for uniform mixing.
9. Cool the solution by keeping the microcentrifuge tube on ice for 5 mins.
10. Spin the tube for 10 min, at ~ 12,000 rpm in microfuge (at 4^0 C)
11. Aspirate and collect the clear supernatant into a fresh 1.5 mL microfuge tube and add equal volumes of isopropanol and invert the tube 20-30 times.
12. Spin the microcentrifuge tube for 10 min, at ~ 12,000 rpm at Room Temparature.
13. Aspirate the supernatant carefully without disturbing the pellet.
14. Re-spin the tube for 5 – 10 sec to collect all residual fluid at bottom of tube.
15. Using a fine-bore pipette tip carefully aspirate and remove the residual fluid.
16. Resuspend the pellet in 50 µL RAS TEB, (In case, dissolved DNA is sticky add 50 After incubation, cool the homogenate RAS TEB more) vortex for ~5 sec, spin briefly to collect liquid at bottom of tube.
17. Heat for ~ 5 min at ~45-55 °C with intermittent vortexing to completely dissolve the nucleic acid.
18. Short Spin the tube to collect the DNA at the bottom and store at -20 °C for further use.

Protocol for DNA Extraction from Blood samples

1. Take 300 µL Blood sample in a microcentrifuge tube and add 1 mL RAS LB and vortex 20 seconds.
2. Place the microcentrifuge tube in ice for 10-15 mins
3. Spin the tube at 6000 rpm for 1 min
4. Discard the supernatant.
5. Repeat the same procedure detailed in Steps 3 to 18 of Protocol for DNA Extraction from Fluids.

Protocol for DNA Extraction from TB Culture from slants and culture plates

1. Take small amount culture using microtip or sterilized inoculation loop.
2. Repeat the same procedure detailed in Steps 3 to 18 of Protocol for DNA Extraction from TB culture from slants and culture plates.

Protocol for DNA Extraction from Liquid TB Culture

1. Take 1 mL of Liquid TB culture in the microcentrifuge tube spin 10,000 rpm for 1min.
2. Discard the supernatant. Wash the pellet twice with MBGW (Molecular Biology Grade Water)
3. Discard the supernatant. Leaving small amount of liquid (approx 100 µL).
4. Repeat the same procedure detailed in Steps 3 to 18 of Protocol for DNA Extraction from Liquid TB cultures.

Protocol for DNA Extraction from Sticky samples (eg. Sputum, Pus)/Decontamination of Sample

For decontamination/sticky solutions, prepare the following solution as described below and follow the following procedure:

Composition for NaLC–NaOH solution

2.9% Sodium Citrate
4N NaOH
0.25 gms N-Acetyl L-Cysteine

Protocol for DNA Extraction from Sticky samples (eg. Sputum, Pus)/Decontamination of Sample

1) Take the sample container and add equal volumes of NaLC-NaOH Solution.
2) Mix properly and keep it at room temperature for 10 min.
3) Transfer 1ml of above mixed solution to 1.5 mL microcentrifuge tube and centrifuge at 10,000rpm for 1min.
4) Discard the supernatant and wash the pellet with sterile water for 2 times.
5) Add 282.8 µL Lysis Mix to the sample.
6) Add 19 µL of RAS LYZ Enzy and incubate at 65 ^{0}C for 10 minutes.
7) After incubation, cool the homogenate and add 10 µL of RAS SDS and 1 µL of RAS PK.
8) Incubate for 1 hour at 65°C in heating block. (Depending upon lysis of sample, incubation time can be reduced)
9) After incubation, cool the homogenate by keeping the microcentrifuge tube in ice for 5 minutes.
10) Add 175 µL of RAS PPS to the homogenate and invert tube 10-20 times for uniform mixing.
11) Cool the solution by keeping the microcentrifuge tube on ice for 5 mins.

12) Spin the tube for 10 min, at ~ 12,000 rpm in microfuge (at 4^0 C)
13) Aspirate and collect the clear supernatant into a fresh 1.5 mL microfuge tube and add equal volumes of isopropanol and invert the tube 20-30 times.
14) Spin the microcentrifuge tube for 10 min, at ~ 12,000 rpm at Room Temparature.
15) Aspirate the supernatant carefully without disturbing the pellet.
16) Wash the pellet once with 70% Ethanol.
17) Re-spin the tube for 5 – 10 sec to collect all residual fluid at bottom of tube.
18) Using a fine-bore pipette tip carefully aspirate and remove the residual fluid.
19) Resuspend the pellet in 50 µL RAS TEB, (In case, dissolved DNA is sticky add 50 After incubation, cool the homogenate RAS TEB more) vortex for ~5 sec, spin briefly to collect liquid at bottom of tube.
20) Heat for ~ 5 min at ~45-55 °C with intermittent vortexing to completely dissolve the nucleic acid.
21) Short Spin the tube to collect the DNA at the bottom and store at -20 °C for further use.

ⓘ If NaLc –NaOH solution has already been used to treat the samples (as followed routinely in Microbiology Labs), then the same sample can be used for DNA extraction. NALC treated sample pelleted and washed with MBGW can be stored at – 20C for 1 month. After centrifugation, wash the pellet twice with sterile water and Repeat the same procedure detailed in Steps 2 to 16 of Protocol for DNA Extraction from Fluids.

Troubleshoot

The most common problem is inhibitors of PCR recovered with the nucleic acid. This can usually be overcome by either using 10-fold less nucleic acid in the PCR, or by using less tissue as starting material in the prep. Another problem is degraded nucleic acid. Degradation is usually due to a long lag time between harvesting the tissue and starting the prep, or due to repeated freeze-thaw of frozen tissue. To avoid degradation, disrupt the tissue as soon as possible after harvesting or store it in a reagent designed to preserve nucleic acid in solid tissue. Alternatively, harvested tissue may be snap-frozen and stored at -70^0C. Storage at -20^0C may result in gradual decline in nucleic acid integrity. Note however that degraded nucleic acid is usually adequate for use as template in PCR, especially for amplicons shorter than ~500 bp.

Technical Assistance

RAS's Technical Service Department is staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of RAS products. If you have any questions or experience any difficulties regarding RAS products in general, please do not hesitate to contact us.

RAS customers are a valuable source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at RAS. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information, please contact our Technical Support Team by email: info@raslifesciences.com

Abbreviations

Abbreviation	***Expansion***
DNA	Deoxy Ribonucleic Acid
BSL2	Bio Safety Level 2
BSL3	Bio Safety Level 3
mL	Milli Liters
µL	Micro Liters
K_2EDTA	Di Potassium Ethylene Diamine Tetra Acetate
MBGW	Molecular Biology Grade Water
Rxn	Reaction
LAMP assay	Loop Mediated Isothermal Amplification assay

Ordering Information

R-DXT-20 : 20 rxns
R-DXT-50 : 50 rxns