prepIT®•MAX

for TB

REF PT-MAX

Laboratory protocol for manual purification of DNA from sputum

The following protocol is a chemical lysis purification method for genomic DNA from sediment samples obtained using OMNIgene•SPUTUM or NaOH/NALC.

Note: This protocol describes a method for purifying DNA from 200 µL of sediment. The volumes can be adjusted for larger sample sizes.

Other languages available at www.dnagenotek.com

CE IVD 15°C 25°C

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Patent (www.dnagenotek.com/legalnotices)
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Intended use

prepIT•MAX is intended for the purification of DNA from sputum obtained from OMNIgene•SPUTUM or NaOH/NALC treatments, in order to release the maximum DNA from *Mycobacterium tuberculosis* (MTb).

Summary and explanation of reagent

prepIT•MAX is a simple and quick extraction method that increases the MTb DNA yield without the need for bead beating or sonication.

Quantity

prepIT•MAX is available in various formats.

Product	Components
PT-MAX-25 (25 preps)	MAX Buffer (5 mL)
	Elution Buffer (5 mL)
	TK Buffer (1 mL)
	MAX Lysis Reagent (1 mL)

Product	Components
PT-MAX-250 (250 preps)	MAX Buffer (50 mL)
	Elution Buffer (50 mL)
	TK Buffer (10 mL)
	MAX Lysis Reagent (10 mL)

Warnings and precautions

For In Vitro Diagnostic Use

1. Clinical specimens which may contain *Mycobacterium tuberculosis* should be considered infectious and handled with appropriate biosafety precautions and standards (follow local and/or federal regulations as appropriate).

   Note: TB bacteria will remain viable in the OMNIgene•SPUTUM reagent.

2. Do NOT use after the "Use by" date indicated on the bottle label.

Storage

prepIT•MAX should be stored at room temperature (15°C-25°C).

Safety information

Wash with water if reagent comes in contact with eyes or skin.
Do NOT ingest.

Safety data sheet (SDS) is available at www.dnagenotek.com

Specimen processing protocol

Reagents included

- MAX Buffer
- Elution Buffer
- TK Buffer
- MAX Lysis Reagent

Equipment and reagents supplied by user

- Centrifuge that is capable of generating 15,000 × *g*
- 1.5 mL microcentrifuge tubes (e.g., Axygen #MCT-150-C)
- Water incubator or hot block at 70°C
- Ethanol (95% to 100%) at room temperature
- Pipettes and pipette tips

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Procedure

The following protocol describes a chemical lysis and genomic DNA purification method from sputum sediments obtained by OMNIgene•SPUTUM or NaOH/NALC treatment.

Purification steps	Notes
1. Remove a 200 µL aliquot of sputum sediment resuspended in sterile phosphate buffered saline (PBS) or water.	The purification protocol can support up to 200 µL of sediment volume in a 1.5 mL microcentrifuge tube and 300 µL of sediment volume in a 2 mL microcentrifuge tube. **Note:** use of phosphate buffer (PB) instead of PBS for sediment resuspension in NOT recommended.
2. Add an equal volume of MAX Buffer.	
3. Add 40 µL (1/10th volume) of MAX Lysis Reagent to the microcentrifuge tube and mix by vortexing for a few seconds.	The 1/10th the volume is calculated according to the total sample volume (e.g., MAX Buffer + sediment volume).
4. Heat at 70°C for 20 minutes.	Shorter incubation time (minimum 5 minutes) may be used, but the DNA yield will be decreased.
5. Add 40 µL (1/10th volume) of TK Buffer to the microcentrifuge tube and mix by vortexing for a few seconds.	The sample will become turbid as impurities and inhibitors are precipitated. The 1/10th the volume is calculated according to the total sample volume (e.g., MAX Buffer + sediment volume).
6. Incubate on ice for 10 minutes or at 4°C for 15 minutes.	
7. Centrifuge at room temperature at 15,000 x *g* for 5 minutes.	
8. Carefully transfer the clear supernatant with a pipette tip into a clean microcentrifuge tube, taking care NOT to disturb the pellet. **Discard pellet containing impurities.**	The pellet contains turbid impurities. If accidentally disturbed, the tube should be re-centrifuged.
9. Add 800 µL room temperature ethanol (95% to 100%). Mix gently by inversion 20 times.	During the mixing with ethanol the sample may become cloudy as DNA precipitates. Even if the sample does NOT become cloudy, DNA will be recovered by carefully following the next steps.
10. Incubate samples at room temperature for 15 minutes to allow the DNA to fully precipitate.	Incubation at -20°C is NOT recommended because impurities may co-precipitate with the DNA.
11. Place the tube in the microcentrifuge in a known orientation. Centrifuge at room temperature for 2 minutes at 15,000 x *g*.	For example, place each tube in the microcentrifuge with the hinge portion of the cap pointing away from the centre of the rotor. After centrifugation, the pellet will be located at the tip of the tube below the hinge. In some cases the pellet may be too small to be easily visible.
12. Carefully remove the supernatant with a pipette tip and discard it. Take care to avoid disturbing the DNA pellet.	This pellet contains DNA; loss of the pellet will result in loss of the DNA. **Note:** The pellet may appear larger than expected due to the presence of residual salt. This salt is NOT inhibitory to downstream molecular applications. Rotating the tube, such that the pellet is on the upper wall, will allow you to safely move a pipette tip along the lower wall and remove all of the supernatant. The supernatant may contain impurities and should be removed completely. Excessive drying of the pellet can make the DNA more difficult to dissolve.
13. Add 100 µL of Elution Buffer to dissolve the DNA pellet. Vortex briefly to fully resuspend DNA.	If a higher concentration of DNA is desired, 50 µL of Elution Buffer should be used. **Note:** large amounts of high molecular weight DNA can be slow to hydrate (dissolve) completely.
14. To ensure complete rehydration of the DNA, incubate at room temperature for 30 minutes. If DNA does NOT go into solution readily, vortex periodically.	Incomplete rehydration of the DNA is a cause of inaccuracy in estimating DNA concentration and potential failure of downstream applications such as PCR.
15. DNA is ready for use in downstream applications.	Options for storage of the fully rehydrated DNA: a) Purified DNA can be stored at room temperature or 4°C for up to 3 months. b) Purified DNA can be frozen in aliquots at -20°C for longer storage.

Shipping

Note: TB bacteria will remain viable in OMNIgene•SPUTUM reagent.

Samples optimized using OMNIgene•SPUTUM reagent should be considered infectious/dangerous goods and travel as UN3373, Biological Substance, Category B.

Packaging and shipment of specimens in OMNIgene•SPUTUM reagent must be done in accordance with local regulations and/or IATA guidelines for the shipment of biohazardous/infectious specimens.

Disposal

Disposal of this product should comply with any local or regional regulations.

Technical support is available Monday to Friday (9h00 to 17h00 EST):

- Toll-free (North America): 1.866.813.6354, option 6
- All other countries: +1.613.723.5757, option 6
- Email: support@dnagenotek.com

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All DNA Genotek protocols, white papers and application notes are available in the support section of our website at www.dnagenotek.com.

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Label legend:

	Use by
CE	CE marking
IVD	In vitro diagnostic medical device
REF	Catalog number
LOT	Lot number
15°C 25°C	Storage instructions
	Manufacturer
EC REP	Authorized representative