For Research Use Only
Not for use in diagnostic procedures
For performance evaluation only

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 (OM-SPD) or NaOH/NALC.

Note: This protocol is described for the purifying of DNA from 200 µL of sediment. The volumes can be adjusted for larger sediment samples.

⚠ 15°C 25°C

Wash with water if liquid solution comes in contact with eyes or skin. Do NOT ingest. See MSDS at www.dnagenotek.com.

Patent (www.dnagenotek.com/legalnotices)

Made in Canada
DNA Genotek Inc.
Ottawa, ON, Canada K2K 1L1
Subsidiary of OraSure Technologies, Inc.

DNA genotek
www.dnagenotek.com

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

Note: TB bacteria will remain viable in OM-SPD reagent.

Intended use

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

This product is intended for Research Use Only, not for use in diagnostic procedures.

Storage

PT-MAX should be stored at room temperature (15°C-25°C) and is stable until the use by date indicated on the bottle label.

Safety information

For further safety information on PT-MAX, refer to the appropriate material safety data sheet (MSDS) available at www.dnagenotek.com.

Reagents included

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

Equipment and reagents supplied by user

- Centrifuge that is capable of generating 15,000 × *g*
- 1.5 mL microcentifuge 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

Procedure

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

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 microcentifuge tube and 300 µL of sediment volume in a 2 mL microcentrifuge tube.
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.

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Purification steps	Notes
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. **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.

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Purification steps	Notes
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. 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. 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.	

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

- Toll-free (North America): 1.866.813.6354, option 6
- All other countries: 613.723.5757, option 6
- Email: support@dnagenotek.com

Some DNA Genotek products may not be available in all geographic regions.
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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.

Label legend:

REF	Catalog number
⚠	Caution, consult instructions for use
15°C 25°C	Storage instructions
	Manufacturer

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