

Materials List for:

A Tuberculosis Molecular Bacterial Load Assay (TB-MBLA)

Wilber Sabiiti¹, Bariki Mtafya^{1,2}, Daniela Alferes De Lima¹, Evelin Dombay¹, Vincent O. Baron¹, Khalide Azam³, Katarina Oravcova⁴, Derek J. Sloan¹, Stephen H. Gillespie¹

¹Division of Infection and Global Health, School of Medicine, University of St Andrews

²National Institute for Medical Research (NIMR)-Mbeya Medical Research Centre

³Instituto Nacional de Saúde (INS), Ministério da Saúde

⁴Institute of Biodiversity, Animal Health & Comparative Medicine, College of Medical, Veterinary & Life Sciences, University of Glasgow

Correspondence to: Wilber Sabiiti at ws31@st-andrews.ac.uk

URL: <https://www.jove.com/video/60460>

DOI: [doi:10.3791/60460](https://doi.org/10.3791/60460)

Materials

Name	Company	Catalog Number	Comments
Heat inactivation:			
15 mL Centrifuge tubes	Fisher-Scientific	10136120	50 mL tubes may be used if larger sample volumes are involved
15 mL Wire Tube rack	Fisher-Scientific	11749128	Other brands with similar make can be used
Water bath	Fisher-Scientific	15700619	Other brands with similar make can be used
RNA extraction:			
Chloroform	Sigma	372978-1L	Not necessary in other RNA extraction kit brands
Ethanol, absolute 99-100%	Sigma	51976-500ML-F	Larger volume available
Extraction control	SOI group St Andrews	VitalbacteriaEC	Supplied with the TB-MBLA Vitalbacteria kit
FASTRNA Pro blue Kit	MP Biomedicals	116025050	Supplied with lysing matrix tubes
Molecular grade water (RNase free)	Sigma	W4502-1L	Qiagen, Fisherscientific can also supply same product
Precellys 24 homogeniser	VWR	432-3750	FastPrep MP Biomedicals can be used too
Refrigerated micro-centrifuge, Fresco 21, Heraeus	Fisher-Scientific	15352117	Other brands with similar capacity can be used
Thermomixer	Eppendorf	BLD-455-010C	Works with 1-2 mL tubes
TURBO DNA-free	Fisher-Scientific	AM1907M	Takes longer but more effective than shorter DNA removal procedures.
RT-qPCR:			
Primers and Taqman probes	SOI group St Andrews	VitalbacteriaPP	Supplied with the TB-MBLA Vitalbacteria kit. Probe fluorophores are FAM (green) for Mtb and HEX (yellow) for Extraction control (EC). Applied Biosystems qPCR platforms use VIC instead of HEX.
QuantiTect Multiplex RT-PCR NR Kit	Qiagen	204845	PCR mix plus the RT enzyme
RotorGene Q 5plex machine	Qiagen	9001580	Other qPCR machines: Vii7, Quantistudio, Steponelus etc. can be used
Strip Tubes and Caps, 0.1 mL	Qiagen	981103	Other tube sizes available depending on the rotor size. For

			other PCR platforms 96 well PCR plates are needed.
Non-heat inactivation Sample preservation materials			
1 M Tris-HCl pH 7.5	Sigma	93313	Supplied ready to use
2-Mercaptoethanol	Sigma	63689	Many competent suppliers
Guanidine thiocyanate (GTC)	Promega	V2791	Promega brand recommended for quality
Molecular grade water (RNase free)	Sigma	W4502-1L	Ensures that GTC solution is free of nucleases
General materials (used but not specific to TB-MBLA)			
500 mL plastic containers		734-5087	Nalgene brand recommended
Biological waste discard jars			Many competent suppliers
Chemical waste discard jars			Many competent suppliers
Disposable gloves, chemical resistant			Many competent suppliers
Freezer (-20 °C)			Many competent suppliers
Freezer (-80 °C)			Many competent suppliers
Fridge (0-8 °C)			Many competent suppliers
Fume hood			For safe handling of toxic reagents
Laboratory scales			For accurate measurement of reagents
Measuring cylinders, plastic			To ensure accurate measurement of reagents
PCR reaction tubes			Should be suitable to the PCR instrument used
Pipettes and matching sterile filtered pipette tips,			DNase and RNase-free, range: P1000, P200, P10, P2 recommended
QIagility loading plates	Qiagen		5-well master mix plate, 16-well reagent plate, 32-well sample plate, 72-well and 96-well reaction plates
QIAGility Pipetting Robot	Qiagen		Important for high throughput pipetting
Racks for 1.5 mL and 2 mL microtubes and for 15 mL and 50 mL Falcon tubes			Chemical-resistant and autoclavable recommended
RNase Away (Removes RNases enzymes from working space)	Fisher Scientific	10666421	Important to ensure services and devices are free from RNase enzyme
Safety goggles, chemical resistant	Fisher Scientific		Can also be got from Sigma. Protect eyes from toxic reagents.
Sterile Pasteur pipettes, 1.5-3 mL			Many competent suppliers
Sterile RNase-free microtubes			1.5 mL tubes suitable for freezing at -80 °C recommended
TB disinfectant, e.g. Tristel Fuse			Ensure it is freshly prepared or prepared within a week
Vortex			Genie 2 brand recommended
Transmission electron microscopy			
Glutaldehyde (8%)	Sigma	G7526-10ML	Part of the Cell fixation buffer (HEPES buffer)

HEPES (pH 7.4, 100 mM)	Sigma	H4034-25G	Part of the Cell fixation buffer (HEPES buffer)
Leica FCS ultracryo	Leica		Cryomicrotome for tissue sectioning
Methyl cellulose	Agar Scientific	AGG6425	Cold mounting resin
Paraformaldehyde (4% in PBS)	Sigma	P6148-500G	Part of the Cell fixation buffer (HEPES buffer)
Sucrose	Sigma	84097-250G	Preservation and mounting medium
Uranyl acetate	Agar Scientific	AGR1260A	Universal Electron microscopy stain