

GenoType[®] Mycobacteria Direct 4.0

Preparation

LB

prewarm to 37°C
check for precipitates; invert if necessary

ICR

ICR + 60 µl CRB = 30 isolations

BB/ICR Mix

100 µl BB + 2 µl ICR = 1 isolation

PC RNA

PC RNA + 15 µl CRB = 3 isolations

MB/BIN Mix

33 µl MB + 220 µl BIN = 1 isolation

PNM^D

11.5 µl PNM + 3.5 µl DMSO = 1 reaction

Enzyme Mix

+ 44 µl EDB

≥ 15 min, RT
do not vortex!

= 8 reactions

-20°C

4 w

LB: Lysis Buffer
ICR: Internal Control RNA
CRB: Control RNA Buffer
BB: Bead Buffer
PC RNA: Positive Control RNA
MB: Magnetic Beads
BIN: Binding Buffer
PNM^D: Primer Nucleotide Mix + DMSO
EML: Enzyme Mix Lyophilisate
EDB: Enzyme Dilution Buffer

A. RNA Isolation

perform in safety cabinet!

230 µl
MB/BIN mix

+ 500-1000 µl sample

perform in safety cabinet!

pipette up and down to mix

15 min, RT

2 min, RT

remove supernatant

+ 50 µl LB

pipette up and down
5 min, RT

+ 150 µl 96% Ethanol

vortex
5 min, RT

2 min, RT
invert

1 min, RT
remove supernatant

+ 1 ml 70% Ethanol

vortex

2 min, RT
invert

1 min, RT
remove supernatant
5 min, RT
remove supernatant

+ 100 µl BB/ICR mix

resuspend
20 min, 85°C without lid

2 min, RT

transfer supernatant

RNA

B. NASBA Reaction

new tube

+ 15 µl PNM^D
+ 10 µl RNA
+ 20-30 µl PO

TwinCubator[®] P2S1
P2S2

TwinCubator[®] P2S3

+ 5 µl Enzyme Mix

TwinCubator[®] P2S4

**use amplification product
for reverse hybridization**