

Microzone Ltd

LYSE 'N EASY™

	<u>Volume</u>
microLYSISPLUS	1 ml
MegaMix-Royal	1 ml
Just water	1 ml

Enough for 50 x DNA release reactions
and 100 x amplifications

Follow 2 stage protocol on a separate sheet

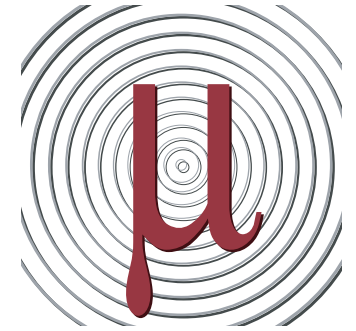
**(Note - microLYSIS-PLUS is a complex solution
which releases (does not purify) the DNA from the
cells. Estimation of DNA yield after lysis can
therefore not be done using a spectrophotometer)**



Store at +4°C

For Research Only

LYSE 'N EASY™



Stage 1 - DNA Release

You need: *micro***LYSIS**[®]-PLUS (MLP) & cells

- Mix cells with 20 μl *micro***LYSIS**[®]-Plus
- Overlay with mineral oil if necessary
- Place in a Thermal Cycler

Lysis Profile

- Step 1: 65°C for 15 mins
(may need to be longer for very tough cells)
- Step 2: 96°C for 2 mins
- Step 3: 65°C for 4 mins
- Step 4: 96°C for 1 min
- Step 5: 65°C for 1 min
- Step 6: 96°C for 30 secs
- Step 7: 20°C hold

After lysis use in **Stage 2**, or store at -20°C for future use



Stage 2 - DNA Amplification



You need: MLP/DNA (MLP-D) from **Stage 1**, Mega**Mix-Royal** (MMR),
Just Water & 2 PCR primers

- Make a premix of the two PCR primers at 2.5 to 5 pmol/μl each
- Make up PCR mastermix as following, adding 1 extra for multiple tubes

	Volume for one	Volume for 4 (x5)
MMR	10 μl	50 μl
Primer Premix	1 μl	5 μl
Just Water	4 μl	20 μl
Total	15 μl	75 μl

- Set up 4 PCRs as follows (guide only)

Tube	1	2	3	4
PCR Mastermix	15 μl	15 μl	15 μl	15 μl
MLP-D	5 μl	2.5 μl	1 μl	- μl
Just Water	- μl	2.5 μl	4 μl	5 μl
Total	20 μl	20 μl	20 μl	20 μl

- Program the Thermal Cycler as follows (guide only)

Initial denaturation step: 95°C for 5 mins

Then cycle 30 times:

Step 1: 95°C for 30 secs

Step 2: Annealing temp of primers for 30 secs

Step 3: 72°C for 45 secs

- After cycling, load 5 μl onto 1 - 2% agarose gel and electrophorese alongside a 100 bp DNA Ladder (not supplied)

Make sure that the sample hasn't evaporated during cycling, as this will distort the results