Instructions for use (v1.5)

#LYSO-25	LysoFAST DNA isolation reagent, 25mL
#LYSO-BOOST-25	LysoFAST BOOST DNA Isolation reagents, 25mL

store at room temperature

Please read the notes at the end of the protocol sheet, as well as the prewash notes below before starting your method.

Prewash

If supplied with your reagent, some applications (e.g. bacteria and yeast in some beer and wine samples, as well as plant samples) can benefit from a prewash step using 100-200 µL of the prewash buffer.

The prewash step is carried out at step 1 or after step 2 (in the applications where samples are first centrifuged.

1. *Resuspend your sample in the prewash buffer before repeating step 1 (in the case of bacteria/yeast).*

Bacteria/Yeast

1. Centrifuge 1-2 mL of sample (e.g. 15,000 x g for 5 minutes) or slower (e.g. 3,000 x g for 10 minutes)
2. Remove all supernatant with a fine-tip transfer pipette (prewash may be performed here – see above)
3. Add 80 µL ready-to-use LysoFAST reagent (if large pellet evident then add 160
4. Vortex samples well and leave on bench for 3-5 minutes (see notes at end).

Samples are ready to PCR. Use 2-3 µL per 20 µL PCR/qPCR

Tissue / Insects

1. Place sample (small or small pieces) in a PCR tube (0.2 mL) or 1.5 mL microcentrifuge tube
2. Add sufficient LysoFAST to cover the sample (30 – 100 µL)
3. Leave on bench for 3-5 minutes (see notes at end). Centrifuge 30 seconds to clarify sample

   Samples are ready to PCR. Use 2-3 µL per 20 µL PCR/qPCR

DNA cards (plant/blood)

1. Place card punch into PCR tube or 1.5 mL tube (depending on size of punch). Prewash may be performed here – see above
2. Add sufficient LysoFAST to cover the sample (30 – 50 µL). Ensure punch is submerged under solution (pulse centrifuge if required)
3. Leave on bench for 3-5 minutes (see notes at end).

   Samples are ready to PCR. Use 2-3 µL per 20 µL PCR/qPCR

Notes

* some samples will benefit from a short heating step e.g. 95°C for 1-5 minutes.

For LysoFAST BOOST, samples should be centrifuged at 12-15,000 x g for 30 seconds immediately prior to adding to amplification reaction. Avoid pipetting any of the resulting pellet.

If DNA is discoloured, then dilute 1:5 in 10mM Tris, pH 8 or TE buffer, pH 8.

If large pellets remain, centrifuge at 12-15,000 x g for 30 seconds and transfer supernatant to new tube.

If testing RNA, run cDNA synthesis reaction or one-step RT-qPCR reaction immediately after incubation time. LysoFAST is compatible with PerfeCTa qPCR ToughMix and qScript™ XLT One-Step RT qPCR Toughmix reagents, as well as repliqa HiFI ToughMix for conventional PCR applications.