Exymes Quick-Start Guide

DNA Extraction Using *bone*GEM™

Ultra Fast DNA Extraction

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*bone*GEM™

This Quick Start Guide provides instructions on extracting DNA from processed bone samples. This protocol is for bone material collected from a sufficiently cleaned bone, i.e. all non-bone tissue and any detritus removed. Bone material should be collected as a powder, ensuring not to overheat the sample during processing. These methods can be further optimised and adapted to suit your sample type. We are available to provide help with developing a custom method.

Please contact us at info@exymesplc.com.

We recommend that you visit: www.exymesplc.com for more information.

General instructions

• All manipulations should be performed in a clean-room or a PCR hood.

• Lab coats, gloves and hairnets should be worn at all times.

• Use only certified DNA-free tubes and reagents.

• Wash any equipment that will come into contact with the sample in 0.05% hypochlorite bleach. Rinse thoroughly with DNA-free water.

Reagent storage

*Bone*GEM reagents are stable at room temperature when shipped but on arrival should be stored at 4°C. After tubes have been opened, the prepGEM and Histosolv should be placed at -20°C. This is to safeguard against accidental contamination and to prevent the degradation of the mesophilic Histosolv enzyme. The buffer can remain at 4°C for convenience.

HISTOSOLV is a mixture of reagents that weakens tissue cells. It is delivered as a lyophilised powder. This should be resuspended in DNA-free water as follows:

Kit size (rxn)	Code	Volume of water to be added
100	BON0100	1.1 ml
500	BON0500	5.5 ml
1000	BON1000	11 ml

Procedure overview

Exymes extraction products use a unique mixture of thermophilic and mesophilic enzymes. Where a low temperature stage is used, the mesophilic Histosolv enzyme comes into play. The 75°C step then activates a thermophilic protease that lyses the cells, kills nucleases and strips the DNA of nucleosomes. During the 75°C step, the Histosolv enzyme is also inactivated. A final 95°C step deactivates the thermophilic proteinase.

Extraction Protocol

The processing of bone material will vary depending on sample type, available equipment, and established protocols. This protocol is currently optimised for bone powder.

1. Place ~10 mg of bone powder into a thin-walled PCR tube.
2. In a clean environment, open the tube and add:

   10 µl of 10x Buffer ORANGE+

   2 µl *bone*GEM

   10 µl Histosolv

   Water to 100 µl
3. Mix the sample by vortexing

   Optional: Improved extraction can be achieved by equilibrating sample at room temperature for 30 minutes. Vortex at 15 minutes.
4. In a thermal cycler, incubate:

   52°C for 10 minutes

   75°C for 20 minutes

   95°C for 5 minutes

   Recommended: Vortex sample during 75°C incubation to resuspend powder.
5. Briefly centrifuge sample to collect powder at the bottom of the tube.
6. Transfer supernatant to a fresh tube. The DNA is in this solution. Do not discard.

For long term storage of the extracted DNA, add one tenth volume 10x TE buffer (100 mM Tris, pH 7.5, 10 mM EDTA). Store at -20°C.

Technical Tips

- *bone*GEM is a preparative method for DNA extraction. The method lyses cells and removes nucleoproteins from the DNA. Extracted DNA can be used for many types of genotyping including SNP and STR analysis as well as quantitative, multiplex and end-point PCR.
- There is no concentration step in the procedure and so the concentration of the extract is dependent on the quality of the sample and the extraction volume.
- There is no purification step in the procedure and using dilutions of the extracted DNA is recommended for optimal results from downstream applications.
- DNA extracted using BoneGEM is largely single stranded because of the 95°C heat step.
- For accurate yield assessment, a qPCR is recommended. If standard fluorescent chelating dyes are to be used for normalising samples, then we recommend taking a sample of the extract before the 95°C step (See Exymes Application Note 109). Alternatively, you can generate a standard curve using a previously made extract that has been quantified.
- As with any preparative method for nucleic acid extraction, best results are obtained when samples are handled at 4°C, or on ice, before and after extraction.
- For long term storage of the extracted DNA, add TE buffer to 1x (10 mM Tris, pH 7.5, 1 mM EDTA) and store at -20°C.

The forensicGEM reagents are stable at room temperature, but after tubes have been opened and for longer term storage, the enzymes should be stored at -20°C and the buffers at 4°C.

More information can be found at
www.exymesplc.com

If you still need help, email us at:
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