MicroGEM Quick-Start Guide

DNA Extraction Using

*forensic*GEM Sperm

MicroGEM

Going to extremes for
effortless DNA extraction

Find more information at
www.microgembio.com

or email
info@microgembio.com

QSG_004_190531_forensicGEM Sperm

*forensic*GEM is validated for forensic DNA extractions. Validation data can be obtained from www.microgembio.com.

General instructions

- All manipulations should be performed in a clean-room or a PCR hood.
- Labcoats, gloves and hairnets should be worn at all times.
- Use only certified DNA-free tubes and reagents.
- Wash equipment that will come into contact with the sample in 0.05% bleach. Rinse thoroughly with DNA-free water.

Acrosolv is a mixture of reagents that weaken tissue cell walls. 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 add
50	FSC0050	0.55 ml
100	FSC0100	1.1 ml
500	FSC0500	5.5 ml
1000	FSC1000	11.0 ml

Reagent storage: *forensic*GEM reagents are stable at room temperature but on arrival should be stored at 4°C. After tubes have been opened, the enzyme and the *Acrosolv* should be placed at -20°C to safeguard against accidental contamination. The buffer can remain at 4°C for convenience.

Procedure overview: MicroGEM extraction products use a unique mixture of thermophilic and mesophilic enzymes. Where a low temperature stage is used, mesophilic cell wall degrading enzymes come into play. The 75° step then activates a thermophilic proteinase that lyses the cells, kills nucleases and strips the DNA of nucleosomes. A final 95° step deactivates the thermophilic proteinase.

Semen

Stains and Swabs

The processing of the sample will vary dependent on sample type. For liquid samples, try to keep the volume of the liquid below 10 µl. With cotton swabs, add 1/4 of the swab directly to the extraction cocktail. Stained fabric can be swabbed or small portions added directly.

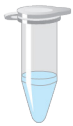
1. Place or pipette the sample into a thin-walled PCR tube

2. Add:
   10 µl 10x **ORANGE+** Buffer
   2 µl *forensic*GEM
   10 µl *Acrosolv*
   Water to 100 µl

3. Mix the sample by vortexing.

4. In a thermal cycler, incubate:
   52°C for 5 minutes
   75°C for 3 minutes
   95°C for 3 minutes

3. Aspirate the extract away from any residual material.



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

- *forensic*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: 1) The quality of the sample; 2) In the case of swabs, the type of swab and the volume of water used to wash the swab; 3) The extraction volume.
- DNA extracted using *forensic*GEM 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. 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 *forensic*GEM 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 is available at www.microgembio.com