

ZyGEM Quick-Start Guide

DNA Extraction Using
forensicGEM™ Sexcrime



Find more information at
www.zygem.com

or email
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See the product documentation on our website.

forensicGEM is validated for forensic DNA extractions. Validation data can be obtained from www.zygem.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 any equipment that will come into contact with the sample in 0.05% hypochlorite 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
100	FSC0100	1.1 ml
500	FSC0500	5.5 ml
1000	FSC1000	11.0 ml

Reagent storage: *ForensicGEM* 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. This is to safeguard against accidental contamination. The buffer can remain at 4°C for convenience.

Procedure overview: ZyGEM 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 of 10x Buffer **ORANGE PLUS**
2 μl *forensicGEM*
10 μl ACROSOLV
Water to 100 μl

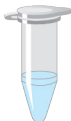


3. Mix the sample by vortexing.

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

- **forensicGEM** 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 **forensicGEM** 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 ZyGEM 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 is available on our website at www.zygem.com