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DNA Extraction Using forensicGEM™ Blood

ZyGEM Quick-Start Guide

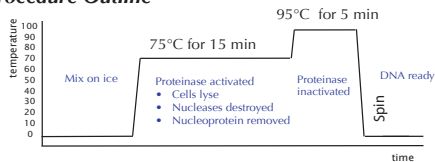
forensicGEM™ Blood

forensicGEM™ is specifically formulated and validated for forensic DNA extractions. Validation data can be obtained from www.zygem.com.

The following method is recommended for extracting DNA from liquid blood using forensicGEM™ Blood.

- All manipulations should be performed in a PCR hood.
- 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.

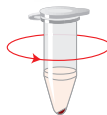
Procedure Outline



Centrifugation Tips

The ZyGEM buffer is a proprietary formulation that precipitates PCR inhibitors. The solid material should not be disturbed when removing the supernatant.

Typically, 2 minutes at 13,000 r.c.f is sufficient to give a well-packed pellet. Longer spins should be used for lower r.c.f. centrifugations. For example, a typical 96-well plate, swing out rotor rated at 3,000 r.c.f. should be spun for 10 minutes.



Extraction Method

- In a thin-walled PCR tube add:
86.5 µl of PCR grade water,
10 µl of 10x Buffer **RED**
2.5 µl of liquid blood
1 µl forensicGEM™

For EDTA stored blood an additional 2 µl of 10 mM CaCl₂ should be added **PURPLE**

- Incubate:
75°C for 15 minutes
95°C for 5 minutes

A thermal cycler can be used for this step

- Centrifuge in a microcentrifuge at full speed for 2-5 min (SEE CENTRIFUGATION TIPS)

- Pipette the supernatant to a new tube without disturbing the pellet

This solution contains the DNA. **Do not discard**

The sample is now ready for analysis. Typically, 5 µl of a 1:10 dilution gives the best results in a PCR
Yields of ~0.5 ng / µl can be expected from fresh blood.

Technical tips for sample management

- forensicGEM™ Blood is a preparative method for DNA extraction. The forensicGEM™ 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 for quantitative, multiplex and end-point PCR.
- DNA extracted using forensicGEM™ is largely single-stranded because of the 95°C heat step.
- For accurate yield assessment, a qPCR is recommended. The DNA produced by forensicGEM™ is approximately 90% single-stranded. If standard fluorescent chelating dyes are to be used for quantification, then this factor should be taken into consideration.
- If extracting DNA from blood containing EDTA, CaCl₂ should be added to a final concentration of 200 µM to improve the enzyme activity.
- 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 4°C but after tubes have been opened and for longer term storage, reagents should be stored at -20°C.
- As with any preparative method for nucleic acid extraction, for best results prepare and manage samples at 4°C, or on ice, before and after extraction.
- When storing the sample after extraction, aspirate the supernatant from the precipitated residue. Be careful not to disturb the pellet.

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