

MicroGEM Quick-Start Guide

DNA Extraction Using

PDQeX

forensicGEM Sperm



Find more information at
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About this Guide

This Quickstart guide provides methods to extract DNA from liquid sperm samples or stains. We are available to provide help with developing any specific method. Please contact us at info@microgembio.com.

Procedure overview: MicroGEM extraction products use a unique mixture of thermophilic and mesophilic enzymes. At 52°C, mesophilic acrosome degrading enzymes come into play. The 75°C step then activates a thermophilic proteinase that lyses the cells, kills nucleases and strips the DNA of nucleosomes. A final 95°C step shrinks the PDQeX tube, bursts the valve and pushes the extract through a column which removes the proteinase and inhibitors

Preparation and Storage of Liquid Reagents

- *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	XSC0050	0.55 ml
100	XSC0100	1.1 ml
500	XSC0500	5.5 ml
1000	XSC1000	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 *forensicGEM* enzyme and the *Acrosolv* should be placed at -20°C to safeguard against accidental contamination. The **ORANGE+** buffer can remain at 4°C for convenience.

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 0.5% bleach. Rinse with DNA-free water.

Precautions

- Do not load the PDQeX machine if the control screen indicates a temperature above 40° C.
- Ensure the collection drawer and heating block are clean and DNA-free.
- Ensure the collection drawer is inserted as far as possible, and that it is straight.
- If fewer than 24 reactions are planned, make sure that the PCR tubes are placed in drawer wells corresponding to the channels to be used in the heating block.

MicroGEM Reagent QC

MicroGEM reagents are manufactured and quality tested following ISO 18385:2016. Our reagents are made from certified DNA-free chemicals and solutions, and all buffers and enzymes are treated with DNase and UV before shipment.

Important Technical Tips

- PDQeX *forensic*GEM Sperm is a method that lyses cells and removes nucleoproteins from the DNA. Extracted DNA can be used for Y-chromosome detection of mixed samples 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 PDQeX *forensic*GEM Sperm is **double-stranded** which means that any fluorescent dye-based quantification such as Pico Green, iQuant, Qubit or the like can be used. With mixed samples, a Y-specific qPCR is recommended.
- 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.

Extraction method

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 PDQeX cartridge. Stained fabric can be swabbed or small portions added directly to the tube.

Preparing the extraction mixture

For each extraction, make up:

10 µl 10x **ORANGE+** Buffer

2 µl *forensicGEM*

10 µl *Acrosolv*

DNA-free water - Add to a final volume of 100 µl

(Note - this will vary depending on whether you add any liquid with your sample).

PDQeX extraction

1. Add the extraction mix with sample into each PDQeX cartridge (100 µl)
2. Put the cap on the PDQeX cartridge by completely inserting the tapered column into the cartridge.
3. Load into the collection drawer either:
 - 24 well plate
 - 8 strip tubes
 - Individual tubes
4. Insert the PDQeX cartridges into the heating block.
5. Put the drawer in place
6. Cover the cartridges with the hinged flap and close the sliding door.

MAKE SURE THE PDQeX CARTRIDGES CORRESPOND WITH A COLLECTION TUBE OR WELL- OTHERWISE YOU WILL LOSE YOUR SAMPLE

7. Select the 'Sperm' program for the extraction:

52°C 5 mins

75°C 3 mins

95°C 2 mins

- **Times may be adjusted by internal laboratory optimisation.**
- **Changes to the default temperatures are not recommended.**