MicroGEM Quick-Start Guide

DNA Extraction Using

*prep*GEM Universal

Find more information at
www.microgembio.com

or email
info@microgembio.com

QSG_prepGEM Universal_20190319

*prep*GEM is for DNA extractions from a range of sample types. More information can be found at 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.

When your kit arrives

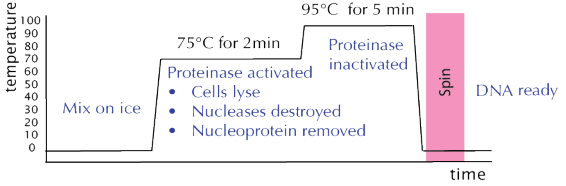
The *Histosolv* is delivered as a dry powder. Add DNA-free water as follows.

Kit size (Rxn)	Code	Volume of water to add
50	PUN0050	0.55 ml
100	PUN0100	1.1 ml
500	PUN0500	5.5 ml
1000	PUN1000	11.0 ml

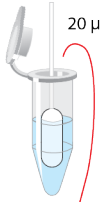
Reagent storage

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

Procedure overview



Buccal swabs



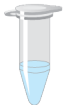
1. Wash the buccal swab in the minimum amount of DNA-free water to cover the swab. Typically, a cotton swab requires 400-500 µl. Use a rolling action against the tube sides and press the swab against the side to squeeze as much of the liquid as possible.

   An alternative approach is to cut off a portion of the swab.

2. In a thin-walled PCR tube add:
   20 µl of the eluate
   10 µl of 10x **BLUE** Buffer
   69 µl of DNA free water
   1 µl of *prep*GEM

Make sure the suspension is agitated prior to adding

3. Incubate at:
   75°C for 5 minutes
   95°C for 2 minutes



Mix before using

DO NOT CENTRIFUGE.

The DNA is high molecular weight and can be sedimented with high speed centrifugation.

The sample is now ready for analysis.

Typically, the method yields DNA at 0.5 - 2 ng / µl depending on the quality of the sampling and the size of the swab.

Tissue Culture

*prep*GEM DNA extraction is ideal for low cell numbers. Because the buffers used for *prep*GEM are compatible with most downstream processes, it means that the entire sample can be used. In addition, because *prep*GEM does not need purifications steps, extractions can be performed in sub-microlitre volumes.

With cultured cells, you can expect linear yields for 5 to approx 100,000 cells and is ideal for single-cell work. For low numbers of cells we recommend reducing the extraction volume. The minimum volume possible will depend on evaporation with the equipment you are using.

The recommended amounts of *prep*GEM to use for different extraction volumes are below. Use 1/10th volume of 10X **BLUE** buffer.

Extraction Volume	Cell numbers	Volume of *prep*GEM
50 - 100 µl	50,000 - 500,000	1 µl
20 - 50 µl	5000 - 50,000	1 µl
5 - 20 µl	100 - 5000	0.5 µl
1 - 15 µl	1 - 500	0.2 µl

Sample handling will vary with different sample types. An outline of some suggested procedures is provided below. More information is available at www.microgembio.com.

Handling different culture types

Cells in suspension

1. Centrifuge the suspension at 200 x g for 5 mins.
2. Remove all of the liquid.
3. Resuspend the pellet in *prep*GEM extraction reagents.

Handling different culture types (continued)

Adherent cells

If the cells are in flasks, dislodge cells by preferred method (Trypsin or cell scraper) and centrifuge suspension at 200 x g for 5 mins. Otherwise, the *prep*GEM reagents can be added directly to the adhered layer.

1. Remove all of the liquid.
2. Add *prep*GEM extraction reagents.

Cells stored in RNAlater™

1. Centrifuge suspension at 3,000 x g for 5 mins.
2. Remove all of the liquid (a quick spin on a bench centrifuge can help to gather the last few drops).
3. Resuspend the pellet in *prep*GEM extraction reagents.

Cell pellets

Up to 5 x 10^5 cells can be extracted using the recommended method. Linear extraction efficiency is best achieved within the range of <10 cells to approximately 10^5. Cell pellets can be used directly. Alternatively, the pellet can be resuspended in 1X **BLUE** buffer and an appropriate quantity added to the extraction.

FACS and LCM

Cells can be collected directly in the extraction reagent mastermix or the reagents added directly to a capillary from LCM. If cells are collected in a different buffer, it may be necessary to add 1/10th volume of the *prep*GEM buffer after collection. We recommend using *prep*GEM reagents within one hour of preparation. For longer periods, reagents should be frozen.

*prep*GEM is sensitive to EDTA and other chelating agents. If cells are presented in EDTA-containing solutions, they should be centrifuged at 200 x g and washed in 1X **BLUE** buffer before use.

Tissue Culture

Extraction (50 µl reaction - can be scaled to any volume)

1. Add:
   Cell suspension or pellet
   5 µl 10x **BLUE** Buffer
   1 µl *prep*GEM
   Water to a final volume of 50 µl

2. Vortex and incubate:
   75 °C for
   - >50,000 cells -10 min
   - 1,000 - 50,000 cells - 5 min
   - <1,000 cells - 2 min

   95 °C for 2 min
   4 °C HOLD

   A thermal cycler can be used for this step.

3. Add 1/10th volume of 10x TE Buffer and store at -20°C or below.

Tissue

Solid Tissue

Cut the tissue into cubes of approximately 1- 2 mm^3. With hair follicles, use 1-3 hairs. Cut off the shaft 4 mm above the follicle.

1. Mix in a thin-walled PCR tube:

   79 µl DNA-free water.
   10 µl of 10x **ORANGE+** Buffer
   1 µl *prep*GEM
   10 µl *Histosolv*

2. Add the sample

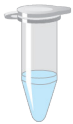
3. Mash the sample with a pipette tip and disperse by vortexing.

4. In a thermal cycler, incubate:

   52 °C for 5 minutes
   75 °C for 10 minutes
   95 °C for 3 minutes

3. Aspirate the extract away from 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.

Insects

Extraction Method

1. Add to the material (volumes can be scaled):
   35 µl of PCR grade water,
   4 µl of 10x **BLUE** Buffer
   1 µl *prep*GEM

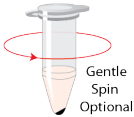
2. Incubate at:
   75 °C for 15 minutes
   95 °C for 2 minutes

A thermal cycler can be used for this step

3. Transfer supernatent to a new tube

The DNA is in this solution. Do not discard. The sample is now ready for PCR.

For storage, at TE to 1x and store at -20°X

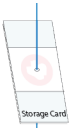


Centrifugation is undesirable for automation and should not normally be needed. However with some material, two minutes at 5,000 x *g* may assist in clarifying the extract. Note: fast spins can sediment genomic DNA.

Depending on the age and quality of tissue, 0.1 - 5 µl of extract is recommended for a 25 µl PCR

Saliva on Storage Cards

Depending on the storage card, it is typical that the preservatives in the card are inhibitory to Taq DNA polymerase and so a pre-wash is recommended prior to DNA extraction



1. Remove one 3 mm disc from the card-stored sample and place into a thin-walled PCR tube or a 96-well plate.

   Uneven application of the swab onto the storage card results in DNA yield variations. For the best results, punch in the centre of the area where the sample was applied.

2. Wash the disk in 100 µl of DNA-Free water by incubating at room temperature for 15 min. Aspirate the water from the disc and discard the water.

3. Add to the tube:

   5 µl of 10x **BLUE** Buffer
   44 µl of DNA-free water
   1 µl *prepGEM*

3. Incubate in a thermal cycler:

   75 °C for 5 minutes
   95 °C for 2 minutes

4. Pipette the solution to a new tube

The DNA is in this solution - not the punch.

The sample is now ready for quantification. Typically, 2 - 5 µl should be used in PCR

Centrifugation Tips

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

Typically, 5 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. Centrifugation should be performed immediately after extraction.

Notes

- Yields will vary depending on the WBC count of the sample.
- Information on how to optimise blood DNA extraction can be found on our website at:

  http://www.microgembio.com/products/prepgem-universal/

- You should be aware that haem coloration carries through to the DNA leaving the sample slightly pink. This does not cause inhibition of PCR, qPCR or human profiling.

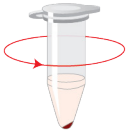
Liquid Blood

Extraction Method

1. In a thin-walled PCR tube add:
   2-5 µl of liquid blood
   10 µl of 10x **RED+** Buffer
   1 µl *prep*GEM
   Add DNA-free water to 100 µl

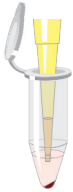
2. In a thermal cycler, incubate:
   75 °C for 5 minutes
   95 °C for 5 minutes

3. Centrifuge in a microcentrifuge at full speed for 5 min



SEE CENTRIFUGATION TIPS

4. Pipette the supernatant to a new tube without disturbing the pellet



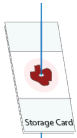
This solution contains the DNA.
Do not discard.

The sample is now ready for use. Typically, 5 µl of a 1:5 dilution gives the best results in a PCR or HID profiling, but depending on your application, we advise testing a few different dilutions.

Yields of ~0.5 ng/µl can be expected from fresh blood.

Blood on Storage Cards

Depending on the storage card, it is typical that the preservatives in the card are inhibitory to *Taq* DNA polymerase and so a pre-wash is recommended prior to DNA extraction.

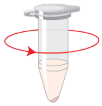


1. Remove one 3 mm disc from the card-stored blood sample and place into a thin-walled PCR tube or a 96-well tray. For the best results, punch in the centre of the area where the blood was applied.

2. Wash the disk in 100 µl of DNA-Free water by incubating at room temperature for 15 min. Aspirate the water from the disc(s) and discard.

3. In a thin-walled PCR tube add:
   5 µl of 10x **RED+** Buffer
   44 µl of DNA-free water
   1 µl *prep*GEM

4. Incubate in a thermal cycler:
   75 °C for 5 minutes
   95 °C for 5 minutes



3. Centrifuge for 2 minutes at maximum speed and transfer the supernatant to a fresh tube (SEE CENTRIFUGATION TIPS)

The DNA is in the solution - not the punch

The sample is now ready for quantification.

Typically, 2 - 5 µl should be used in PCR

Technical Tips

- *prep*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 (which in some cases can be scaled).

- DNA extracted using *prep*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.

*prep*GEM reagents are stable at room temperature, but after the 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.microgembio.com

If you still need help, email us at:
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