

ZyGEM Quick-Start Guide

DNA Extraction Using *prepGEM™* Universal



Find more information at
www.zygem.com

or email
info@zygem.com

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prepGEM is for DNA extractions from a range of sample types. More information can be obtained from www.zygem.com.

General instructions

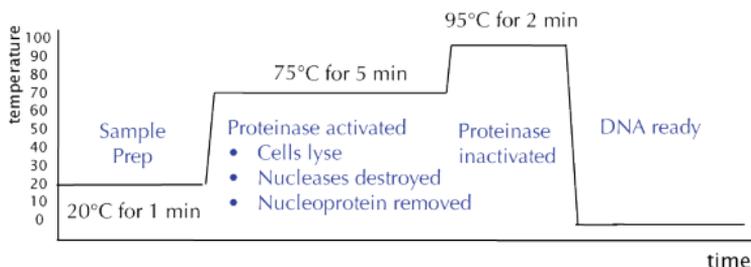
- All manipulations should be performed in a clean-room or a PCR hood.
- Labcoats, gloves and hairnets should be worn at all times.
- Use only certified DNA-free tubes and reagents.
- Wash any equipment that will come into contact with the sample in 0.05% hypochlorite bleach. Rinse thoroughly with DNA-free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

Reagent storage

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

Saliva / Tissue Procedure overview

ZyGEM extraction products use a unique thermophilic proteinase. Extractions are performed at 75°C and the proteinase is inactivated at 95°C. A thermal cycler is used for this process.



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. This method is described in our Application Note 106.



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

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

prepGEM DNA extraction is ideal for low cell numbers. Because the buffers used for *prepGEM* are compatible with most downstream processes, it means that the entire sample can be used. In addition, because *prepGEM* 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 *prepGEM* to use for different extraction volumes are below. Use 1/10th volume of 10X **BLUE** buffer.

Extraction Volume	Cell numbers	Volume of <i>prepGEM</i>
50 - 100 μ l	50,000 - 500,000	1 μ l
20 - 50 μ l	5000 - 50,000	1 μ l
5 - 20 μ l	100 - 5000	0.5 μ l
1 - 15 μ l	1 - 500	0.2 μ l

Sample handling will vary with different sample types. An outline of some suggested procedures is provided below. More information is available at www.zygem.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 *prepGEM* extraction reagents.

Tissue Culture

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 ZyGEM reagents can be added directly to the adhered layer.

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

Cell pellets

Up to 5×10^5 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 ZyGEM buffer after collection. We recommend using ZyGEM reagents within one hour of preparation. For longer periods, reagents should be frozen.

prepGEM 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 μ l reaction - can be scaled to any volume)

1. Add:

Cell suspension or pellet

5 μ l 10x Buffer **BLUE**

1 μ l prepGEM

Water to a final volume of 50 μ l

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 mm³. With hair follicles, use 1-3 hairs. Cut off the shaft 4 mm above the follicle.



1. Mix in a thin-walled PCR tube:
 - 71 μ l DNA-free water.
 - 10 μ l of 10x Buffer **ORANGE PLUS**
 - 1 μ l *prepGEM*
 - 10 μ l HISTOSOLV

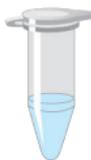


2. Add the sample
3. Mash the sample with a pipette tip and disperse by vortexing.



4. In a thermal cycler, incubate:
 - 52°C for 5 minutes
 - 75°C for 5 minutes
 - 95°C for 3 minutes
3. Aspirate the extract away from residual material.

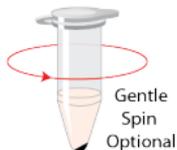
The DNA is in this solution. Do not discard.



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. Place the sample in a tube and grind.
 2. Add to the material (volumes can be scaled):
35 μ l of PCR grade water,
4 μ l of 10x Buffer
1 μ l prepGEM
 3. Incubate at:
75°C for 15 minutes
95°C for 2 minutes
- A thermal cycler can be used for this step
3. Give the tube a low speed spin to sediment any solids
 4. Transfer supernatant to a new tube

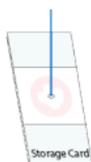
The DNA is in this solution. Do not discard.
The sample is now ready for PCR.

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

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.

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 using the punch provided with the kit 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 Buffer **BLUE**

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

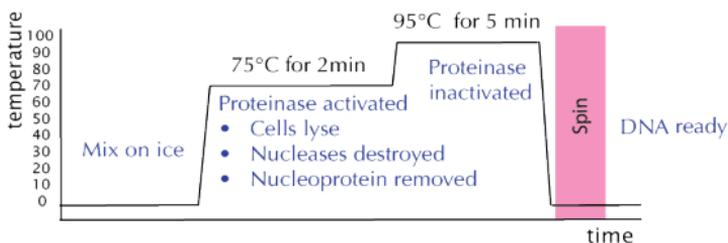
4. Pipette the solution to a new tube

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

Blood Methods

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, 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.zygem.com/support/application-notes>

- 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

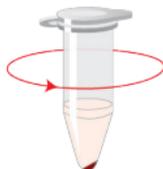
1. In a thin-walled PCR tube add:
2-5 μl of liquid blood
10 μl of 10x Buffer **RED PLUS**
1 μl *prepGEM*
DNA-free water to 100 μl



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.



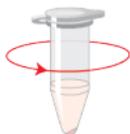
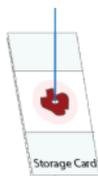
The sample is now ready for use. Typically, 5 μl of a 1:10 dilution gives the best results in a PCR or HID profiling, but depending on your application, we advise testing a few different dilutions.

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

Please visit www.zygem.com for more information

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 using the punch provided 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 μ l 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 μ l of 10x Buffer **RED PLUS**
 - 44 μ l of DNA-free water
 - 1 μ l *prepGEM*
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 μ l should be used in PCR

Technical Tips

- *prepGEM* 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 *prepGEM* 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 *prepGEM* 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 can be found at :
www.zygem.com

If you still need help, email us at:
info@zygem.com

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