eXymes Quick-Start Guide

DNA Extraction Using

*prep*GEM™ Universal

Ultra Fast DNA Extraction

Find more information at
www.exymesplc.com

or email
info@exymesplc.com

*prep*GEM™

*prep*GEM is for DNA extractions from a range of sample types. More information can be obtained from www.exymesplc.com

General instructions

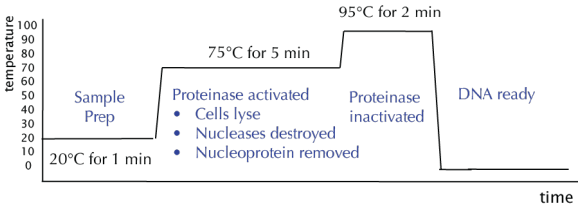
- All manipulations should be performed in a clean-room or a PCR hood.
- Labcoats, gloves and hairnets should be worn at all times.
- Use only certified DNA-free tubes and reagents.
- Wash any equipment that will come into contact with the sample in 0.05% hypochlorite bleach. Rinse thoroughly with DNA-free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

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.
   10µl of 10x Buffer **BLUE**
   69µl of DNA free water
   1 µl *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.exymesplc.com.

Handling different culture

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.

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 eXymes 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 eXymes buffer after collection. We recommend using eXymes 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

Tissue Culture

Extraction (50 µl reaction - can be scaled to any vol-

1. Add:
   - Cell suspension or pellet
   - 5 µl 10x Buffer **BLUE**
   - 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 10xTE Buffer and store at -20°C or below.

Tissue

Solid Tissue

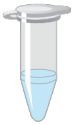
Cut the tissue into cubes of approximately 1- 2 mm^3. With hair follicles, use 1-3 hairs. Cut off the shaft 4 mm above the follicle.

1. Mix in a thin-walled PCR tube:
   - 71 µl DNA-free water.
   - 10 µl of 10x Buffer ORANGE PLUS
   - 1 µl prepGEM
   - 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 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 ina tube and grind.
2. Add to the material (volumes can be scaled):
   35 µl of PCR grade water,
   4 µl of 10x **BLUE** Buffer
   1 µl prepGEM
3. Incubate at: 75°C for 15 minutes
   95°C for 2 minutes

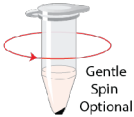
A thermal cycler can be used for this step

3. Give the tube a low speed spin to sediment any solids

4. Transfer supernatent to a new tube

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

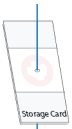
For storage, at TE to 1x and store at -20°C



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

Saliva on Storage

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:

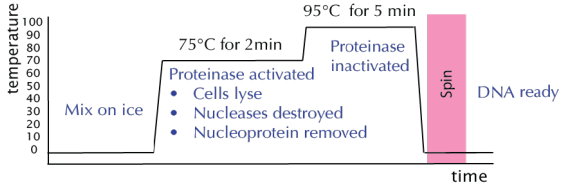
   75°C for 5 minutes
   95°C for 2 minutes

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 eXymes 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 wellpacked 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.exymes.com

- 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.

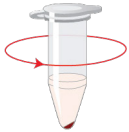
Liquid Blood

Extraction Method

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

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

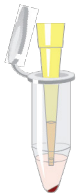
   95°C for 5 minutes



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 pelletThis solution contains the DNA. Do not discard.



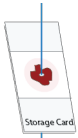
The sample is now ready for use. Typically, 5 µl of a 1:10 dilution gives the best results in a PCR or HID profiling, but depending on your application, we advise testing a few different dilutions.

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

Please visit www.exymesplc.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 prewash 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 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 Buffer RED PLUS
   44 µl of DNA-free water
   1 µl *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 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 (See eXymes 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 *prep*GEM 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.exymesplc.com

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