Exymes Quick-Start Guide

DNA Extraction Using *forensic*GEM™ Universal

Exymes

Ultra Fast DNA Extraction

Find more information at
www.exymesplc.com

or email
info@exymesplc.com

QSG003

*forensic*GEM™

*forensic*GEM is validated for forensic DNA extractions. Validation data 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.

HISTOSOLV 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
100	FUN0100	1.1 ml
500	FUN0500	5.5 ml
1000	FUN1000	11.0 ml

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

Procedure overview: Exymes extraction products use a unique mixture of thermophilic and mesophilic enzymes. Where a low temperature stage is used, mesophilic cell wall degrading enzymes come into play. The 75° step then activates a thermophilic proteinase that lyses the cells, kills nucleases and strips the DNA of nucleosomes. A final 95° step deactivates the thermophilic proteinase.

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 *forensic*GEM

   Make sure the suspension is agitated prior to adding

3. In a thermal cycler, incubate:
   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

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 Buffer **ORANGE PLUS**
   1 µl *forensic*GEM
   10 µl HISTOSOLV

2. Add the sample

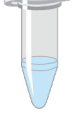
3. Mash the sample with a pipette tip and disperse by vortexing.

4. In a thermal cycler, incubate:

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

3. Aspirate the extract away from residual material.

The DNA is in this solution. Do not discard.



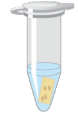
For long term storage of the extracted DNA, add one tenth volume 10x TE buffer (100 mM Tris, pH 7.5, 10 mM EDTA). Store at -20°C.

Cigarette Butts

Cigarette butts are difficult to process because of the release of tars and phenolics that can inhibit *Taq* DNA polymerase. The *forensic*GEM method resolves this problem by using a gentle lysis which extracts the DNA without releasing the inhibitors.

1. Remove a 1 cm strip of paper from the cigarette butt and cut into quarters. These can be processed separately as replicates.
2. Cut each sample of paper into smaller pieces (3 mm squares) and place in a thinwalled PCR tube or 96-well PCR tray.

3. Add:
   44 µl DNA-free water.
   5 µl of 10x Buffer **BLUE**
   1 µl *forensicGEM*
4. In a thermal cycler, incubate:
   75°C for 5 minutes
   95°C for 2 minutes



5. Aspirate the extract away from the paper immediately and transfer to a fresh tube.

The DNA is in this solution. Do not discard.

The paper can be discarded.

Please see the cigarette application note at:

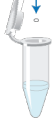
http://www.exymesplc.com

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 prewash 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:

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

4. In a thermal cycler, incubate:

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

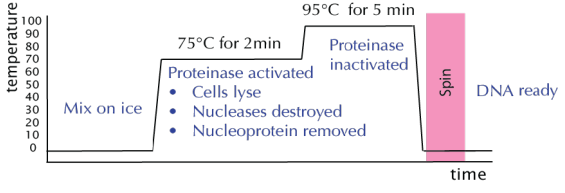
5. Pipette the solution to a new tube

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 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.exymesplc.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:
   2-5 µl of liquid blood
   10 µl of 10x Buffer RED PLUS
   1 µl *forensic*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 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.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 *forensicGEM*

4. In a thermal cycler, incubate:
   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

Bloodstains

You can use the same method for the storage card with bloodstains. Methods will vary depending on the sample type.

Direct extraction

1. Cut a piece of stained fabric no bigger than 3 mm x 3 mm. And place in a thin-walled PCR tube.
2. Add:
   5 µl of 10x Buffer RED PLUS
   4 µl of DNA-free water
   1 µl *forensicG*EM
3. In a thermal cycler, incubate:
   75°C for 5 minutes
   95°C for 5 minutes
4. Centrifuge for 2 minutes at maximum speed and transfer the supernatant to a fresh tube

   (SEE CENTRIFUGATION TIPS)

Wet swab lift

1. Lift the blood from the stain using a damp swab. Small foam swabs are preferred.
2. To a thin-walled PCR tube, add:
   10 µl of 10x Buffer
   89 µl of DNA-free water
   1 µl *forensicGEM*
3. Wash the swab in the extraction solution using a rlling action on the sides of the tube. Squeeze as much liquid as possible out of the swab.
4. In a thermal cycler, incubate:
   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)

Technical Tips

- *forensic*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 *forensic*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 *forensic*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 is available on our website at www.exymesplc.com