*forensic*GEM Handbook

*forensic*GEM Universal | *forensic*GEM Sperm

Exymes

C0084 V1

CONTENTS

KIT CONTENTS AND STORAGE

Exymes kits come with proprietary buffers and enzymes required for nucleic acid extraction, listed in Table 1 and Table 2. MicroGEM kits contain excess volume to allow for minor pipetting errors and over-age while preparing the Master Mix for extraction ensuring customers get the full number of reactions out of their kits.

*forensic*GEM Universal Kit Contents

Kit contains: forensicGEM, 10X BLUE buffer, 10X RED+ buffer, 10X ORANGE+ buffer, Histosolv

Table 1. Kit components for forensicGEM Universal.

Component	Volumes				Temperature (shipping)	Temperature (storage)
	50 rxn	100 rxn	500 rxn	1,000 rxn		
Catalogue no.	PUN0050	PUN0100	PUN0500	PUN1000		
forensicGEM*	50 µl	100 µl	500 µl	1000 µl	RT	-20°C
10X BLUE buffer	1 ml	1 ml	5 ml	10 ml	RT	4°C
10X RED+ buffer	1 ml	1 ml	5 ml	10 ml	RT	4°C
10X ORANGE+ buffer	1 ml	1 ml	5 ml	10 ml	RT	4°C
Histosolv**	50 rxn	100 rxn	500 rxn	1,000 rxn	RT	-20°C

*NOTE: After tubes have been opened, the forensicGEM enzyme should be placed at -20°C. To minimize the number of freeze/ thaw cycles, forensicGEM can be aliquoted into smaller volumes.

**NOTE: Once Histosolv has been rehydrated, it is stable 7-12 months at -20°C. If you do not plan to use all the Histosolv immediately, it is recommended that you aliquot Histosolv into smaller volumes and store at -20°C to minimize the number of freeze/thaw cycles.

*forensic*GEM Sperm Kit Contents

Kit contains: forensicGEM, 10X ORANGE+ buffer, Acrosolv

Table 2. Kit components for forensicGEM Sperm.

Component	Volumes				Temperature (shipping)	Temperature (storage)
	50 rxn	100 rxn	500 rxn	1000 rxn		
Catalogue no.	FSC0050	FSC0100	FSC0500	FSC1000		
forensicGEM*	100 µl	200 µl	1000 µl	2x1000 µl	RT	-20°C
10X ORANGE+ buffer	1 ml	1 ml	5 ml	10 ml	RT	4°C
Acrosolv**	50 rxn	100 rxn	500 rxn	1000 rxn	RT	-20°C

*NOTE: After tubes have been opened, the forensicGEM enzyme should be placed at -20°C. To minimize the number of freeze/ thaw cycles, forensicGEM can be aliquoted into smaller volumes.

**NOTE: Once Acrosolv has been rehydrated, it is stable 7-12 months at -20°C. If you do not plan to use all the Acrosolv immediately, it is recommended that you aliquot Acrosolv into smaller volumes and store at -20°C to minimize the number of freeze/thaw cycles.

Storage

Once received MicroGEM Kit buffers and reagents should be stored dry at the temperatures indicated on page 3 in Table 1 and Table 2. forensicGEM must be stored at -20°C, potentially in aliquots to reduce the number of freeze/thaw cycles. Histosolv and Acrosolv once hydrated, should also be stored at -20°C in aliquots if not planning on using immediately to minimize the number of freeze/thaw cycles. Buffers should be stored at 4°C.

PRODUCT INFORMATION

Product Use Limitations

Exymes kits are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease. All due care and attention should be exercised in the handling of the products. We recommend all users of Exymes products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines. *NIH Office of Science Policy:* https://osp.od.nih.gov/biotechnology/biosafety-and-recombinant-dna-activities/

Product Warranty and Satisfaction Guarantee

Exymes guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, Exymes will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a Exymes product does not meet your expectations, simply contact:

Technical Service (techsupport@exymesplc.com) or their distributor.

We will credit your account or exchange the product. A copy of Exymes terms and conditions can be obtained on request and is also provided on the back of our invoices. If you have questions about product specifications or performance, please contact Technical Service (techsupport@exymesplc.com) or your distributor.

Technical Assistance

At Exymes, we pride ourselves on the quality and availability of our technical support. Our Technical Support Department is staffed by experienced scientists with extensive practical and theoretical expertise in sample and assay technologies and the use of Exymes products. If you have any questions or experience any difficulties regarding *forensic*GEM Kits or Exymes products in general, please do not hesitate to contact us at:
techsupport@exymesplc.com

Exymes customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at Exymes. We therefore encourage you to contact us if you have any suggestions about product performance, publications or new applications and techniques.

Quality Control

Exymes reagents are made from certified DNA-free chemicals and solutions and all buffers and enzymes are treated with DNase and UV before shipment. Be aware however, that we have no control over the reagents of other vendors.

Safety Information

For safety information, please consult the appropriate safety data sheets (SDSs). These are available online in the resource section of the product pages. These are available online in the resource section of the product pages or by emailling techsupport@exymesplc.com.

Product Principle/Product Overview

Exymes's extraction process relies on temperature-driven extraction utilizing a thermostable proteinase, which functions at temperatures ideal for nucleic acid extraction – inactive at low temperatures, optimally active at 75°C, lysing cells, and removing nucleoproteins from the DNA, and inactivating again at 95°C. This 95°C inactivation step results in DNA that is largely single-stranded, thus still suitable for many applications such as: genotyping including SNP and STR analysis, as well as PCR, and qPCR. This process reduces the number of steps required compared to traditional extraction methods (i.e., silica columns, magnetic beads, or organic extractions) reducing time and increasing DNA yield through no loss during the extraction process. The Exymes *forensic*GEM Universal kit is designed for extraction of DNA from a variety of sample types. This includes, but is not limited to saliva (liquid, swab, stains, and storage card-FTA), blood (liquid, swab, stains, and storage card-FTA), buccal swabs and tissue (solid, hair). For sperm and semen samples, Exymes's *forensic*GEM Sperm Kit (FSC) can extract DNA from tough sperm heads without the need for reducing agents such as SDS, mercaptoethanol or Dithiothreitol (DTT), which have been shown to inhibit qPCR and PCR analysis[1] .

Procedure Overview (*forensic*GEM Universal)

Workflow for extraction from sample to DNA in < 15 min 1 to 96 samples.

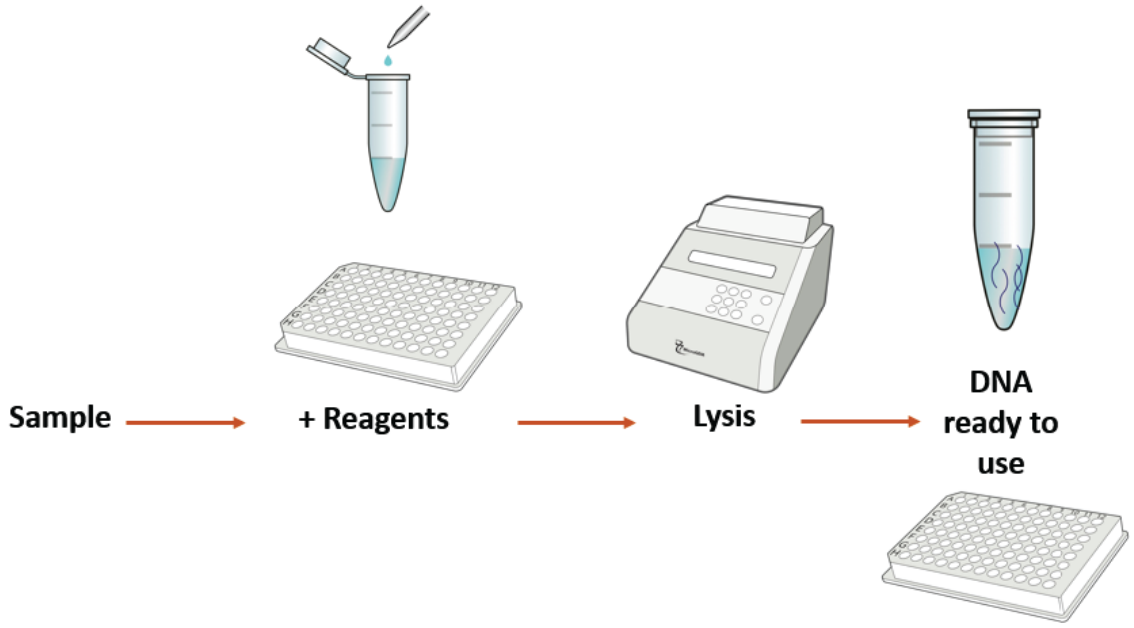


Figure 1.
Generalized workflow for sample processing (1-96 samples) using MicroGEM' s temperature driven extraction (TDE) process.

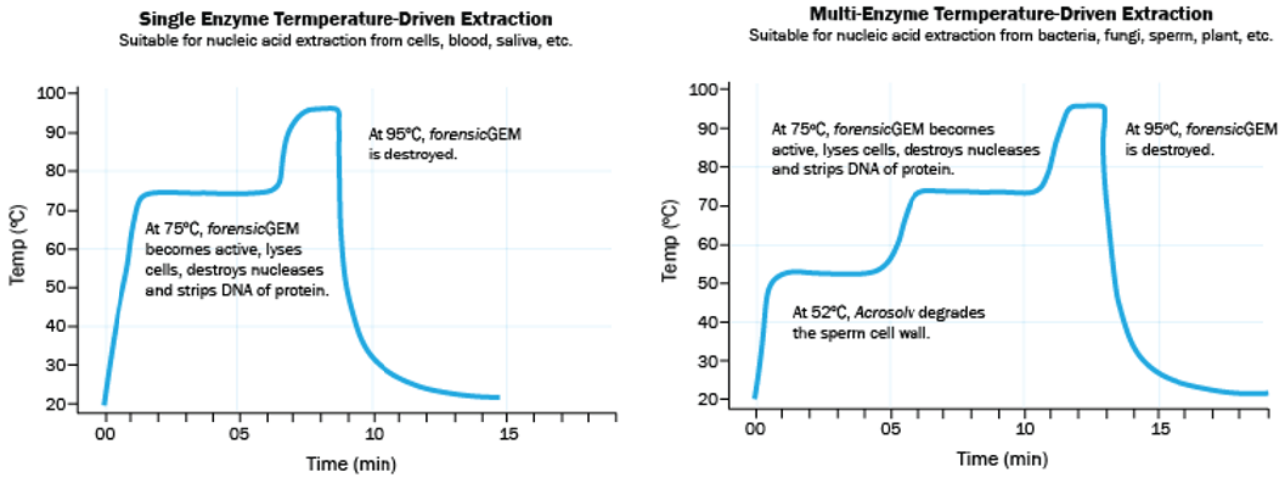


Figure 2.
(Left) for single enzyme Temperature Driven Extraction (TDE), forensicGEM is the only enzyme in the reaction. Simple temperature changes activate the enzyme to extract DNA, free of proteins. (Right) Multi-enzyme TDE utilizes multiple enzymes [e.g., Histosolv (*forensic*GEM Universal – tissue samples) or Acrosolv (*forensic*GEM Sperm) to extract DNA from more challenging sample types]. The low activity of the *forensic*GEM enzyme below 75°C allows for mesophilic cell-wall degrading enzymes can be used when needed for specific sample types. Both reactions can be carried out in a single tube or scaled up to a 96-well plate.

Equipment and Reagents to be Supplied by User

For all protocols:

Equipment

Vortexer

Pipettes

Thermocycler or heat block

Consumables

Pipette tips

Nuclease-free water

Nuclease-free (RNase and DNase Free) microfuge tubes/plates

TE-1 buffer (not necessary, but for long term DNA storage)

Technical Tips

- The method, enzyme formulation and buffer have been carefully optimized for extracting DNA. Using the enzyme with other methods or buffers is not recommended. If you need to modify the method in any way, please email: techsupport@exymesplc.com
- forensicGEM is a preparative method for DNA extraction. This method lyses cells and removes nucleoproteins from the DNA. Extracted DNA can then be used for many molecular biology applications 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 input; 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).
- The laboratory shall have separate work areas with dedicated equipment and supplies for pre- and post-PCR activities to reduce the risk of introducing amplified DNA into samples.
- DNA extracted using forensicGEM is largely single stranded because of the 95°C heat step.
- For accurate quantification, a quantitative PCR (qPCR) is recommended. If standard fluorescent chelating dyes are to be used for normalizing 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. Additional quantification information can be found on the Exymes website.
- As with any preparative method for nucleic acid extraction, best results are obtained when samples are fresh, and handled at 4°C, or on ice, before and after extraction.
- For long term storage of the extracted DNA, add TE buffer and store at -20°C.

PRE-EXTRACTION STEPS

Pre-Extraction (forensicGEM Universal): Resuspend the Histosolv

Histosolv is delivered as a dry powder. Before it is ready to be use, the powder should be dissolved in Nuclease-free water. Different kit sizes contain tubes with different amounts of enzyme (ensure you follow the directions on the Histosolv label in your kit). Be sure to add the correct amount of water (see the table below).

1. In a clean environment, open the tube and add:

Kit reaction size	Product Code	Nuclease-free water
50 rxn	FUN0050	0.55 ml
100 rxn	FUN0100	1.1 ml
500 rxn	FUN0500	5.5 ml
1000 rxn	FUN1000	11.0 ml

2. Once Histosolv has been rehydrated it is stable for 7-12 months at -20°C. If you do not plan to use all of the Histosolv immediately, it is recommended that you aliquot Histosolv into smaller volumes and store at -20°C immediately after rehydration.

Pre-Extraction (forensicGEM Sperm): Resuspend the Acrosolv

Acrosolv is delivered as a dry powder. Before it is ready to be use, the powder should be dissolved in Nuclease-free water. Different kit sizes contain tubes with different amounts of enzyme (ensure you follow the directions on the Acrosolv label in your kit). Be sure to add the correct amount of water (see the table below).

Kit reaction size	Product Code	Nuclease-free water
50 rxn	FSC0050	0.55 ml
100 rxn	FSC0100	1.1 ml
500 rxn	FSC0500	5.5 ml
1000 rxn	FSC1000	11.0 ml

3. Once *Acrosolv* has been rehydrated it is stable for 7-12 months at -20°C. If you do not plan to use all of the *Acrosolv* immediately, it is recommended that you aliquot Acrosolv into smaller volumes and store at -20°C immediately after rehydration.

Prepare Extraction Master Mix

In order to ensure that the yields are uniform amongst samples, it is recommended that an extraction Master Mix be prepared prior to performing extractions. Master Mix can then be either added to sample in tubes or put into tubes prior to adding sample. An example of a Master Mix preparation is shown below:

Table 3.
Example calculation for preparing Master Mix for 10 buccal swab (eluate) samples

Component	Volume per single extraction (µl)	Number of Reactions +1 (overage)	Total volume added (µl)
10X BLUE buffer	10	11	110
Nuclease-free water	69	11	759
forensicGEM	1	11	11

*forensic*GEM UNIVERSAL PROTOCOLS

DNA extraction from buccal swabs

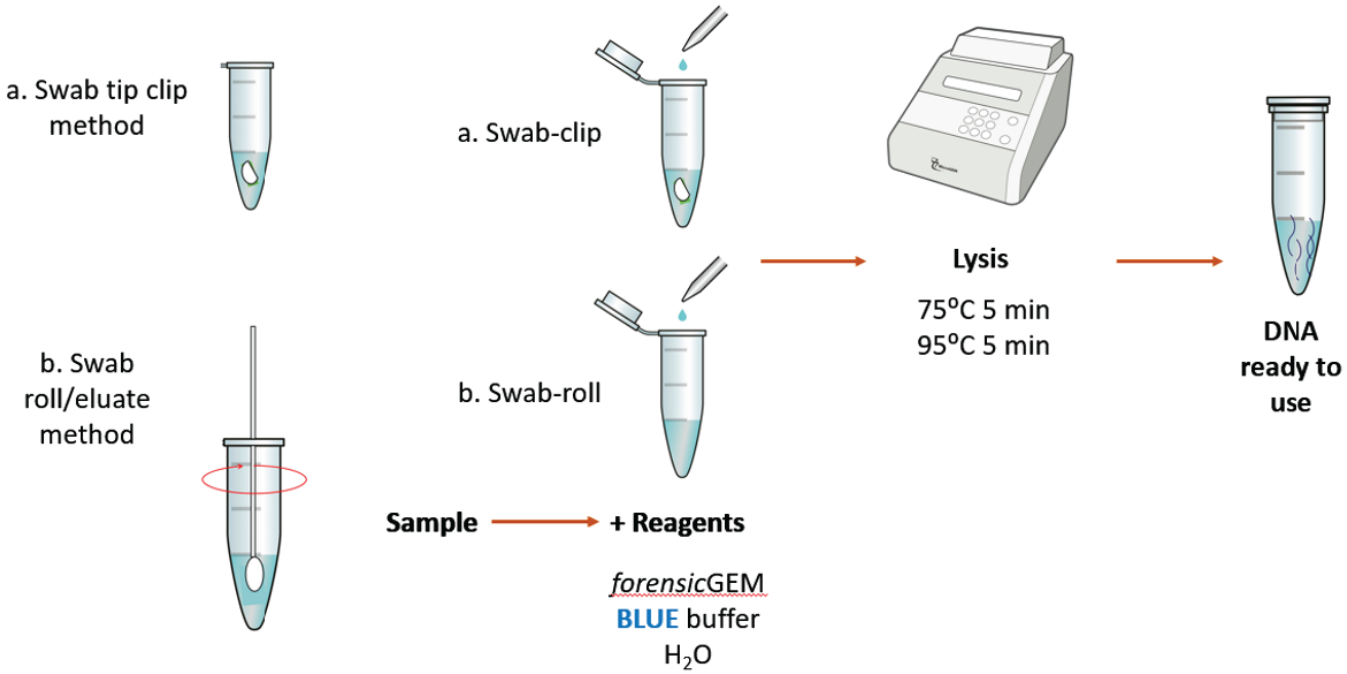


Figure 3. Workflow for DNA extraction from buccal swabs (a) swab tip clip method (b) swab roll/eluate method.

Buccal swab tip clip method (a)

1. Clip the tip of the buccal swab off and into a thin-walled PCR tube or 96-well plate.

2. To the PCR tube containing the buccal swab tip, add:

    a. 10 µl of the 10X BLUE buffer
    b. 1 µl *forensic*GEM
    c. 89 µl Nuclease-free water

3. Vortex each sample for 5 seconds. Pulse spin in a mini centrifuge to remove droplets from the lid and walls of the tube.

4. Place the samples in a thermocycler and incubate for the following temperatures and times:
    a. 75°C for 5 min
    b. 95°C for 5 min

5. Pulse spin in a mini centrifuge to remove droplets from the lid and walls of the tube.

6. The sample is now ready for analysis. Vortex and pulse spin before using.

Buccal swab roll/eluate method (b)

1. Wash the buccal swab in a minimum amount of Nuclease-free water to cover the swab head. Typically, a cotton swab requires 400-500 µl. Less volume can be used if using less swab material. Use a rolling action against the tube sides and press the swab against the side to squeeze as much of the liquid out as possible. Remove 20 µl of eluate from this solution and place into a thin-walled PCR tube or 96-well plate.

2. To the PCR tube containing the eluate (20 µl), add:
    a. 10 µl of the 10X BLUE buffer
    b. 1 µl *forensic*GEM
    c. 69 µl Nuclease-free water

3. Vortex each sample for 5 seconds. Pulse spin in a mini centrifuge to remove droplets from the lid and walls of the tube.

4. Place the samples in a thermocycler and incubate for the following temperatures and times:
    a. 75°C for 5 min
    b. 95°C for 5 min

5. Pulse spin in a mini centrifuge to remove droplets from the lid and walls of the tube.

6. The sample is now ready for analysis. Vortex and pulse spin the extract before using.

DNA extraction from cigarette butts or other suspected saliva stains (forensicGEM Universal kit, BLUE buffer)

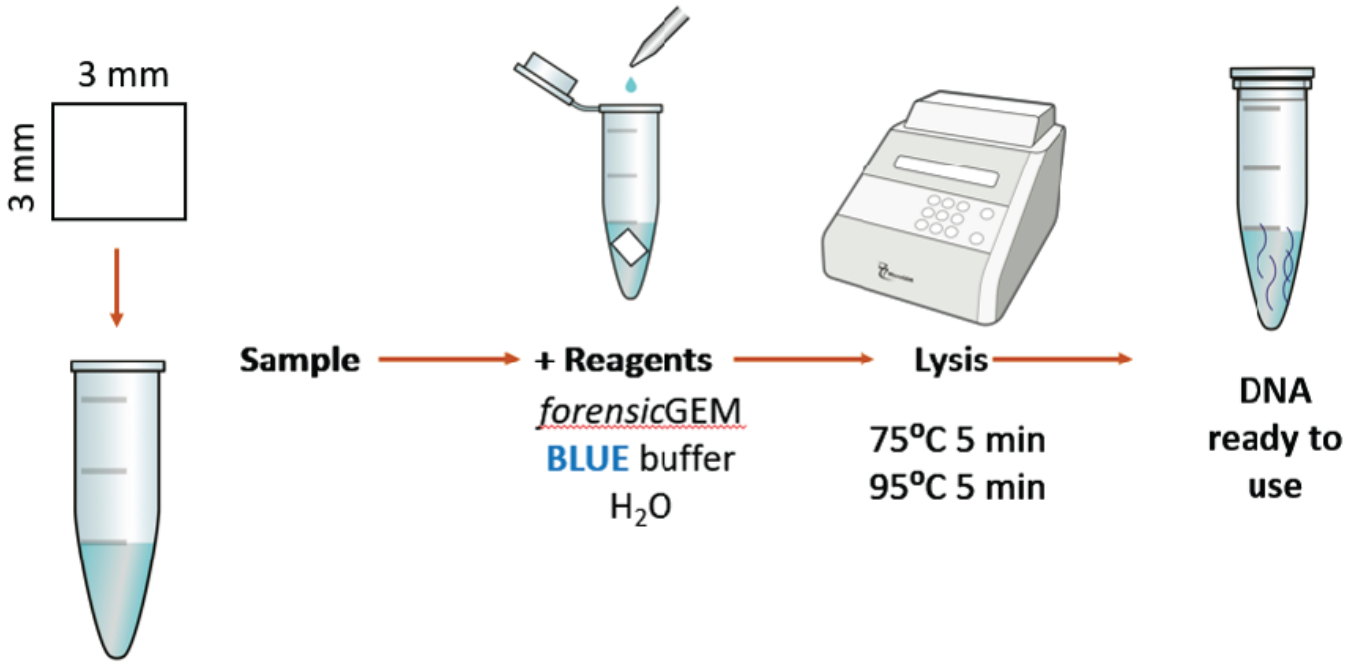


Figure 4. Workflow for cigarette butt/non-porous sample extraction.

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

1. Remove the paper from the cigarette butt and cut out a 3mm² section place into a thin-walled PCR tube or 96-well plate

2. To the PCR tube containing the paper, add:
    a. 5 µl of the 10X BLUE buffer
    b. 1 µl *forensic*GEM
    c. 44 µl Nuclease-free water

3. Vortex each sample for 5 seconds. Pulse spin in a mini centrifuge to remove droplets from the lid and walls of the tube.

4. In a thermocycler, incubate:
    a. 75°C for 5 min
    b. 95°C for 5 min

5. Aspirate the extract away from the paper.

6. The sample is in the solution (DO NOT DISCARD) and ready for analysis. The paper can be discarded. Vortex and pulse spin the extract before using.

DNA extraction from saliva on storage cards (forensicGEM Universal kit, BLUE buffer)

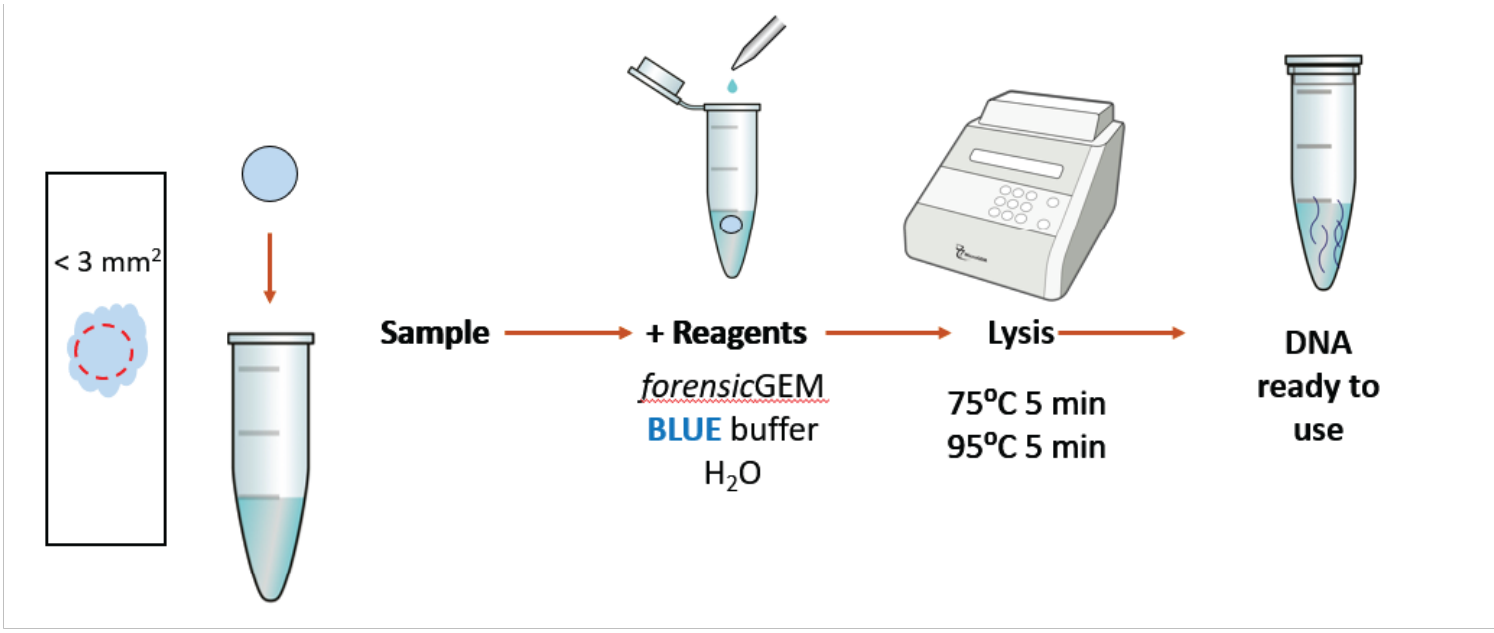


Figure 5. Workflow for DNA extraction from saliva on storage cards.

Depending on the storage card, it is possible 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.

2. Wash the disc in 100 µl of Nuclease-free water by incubating at room temperature for 15 minutes.

3. Aspirate the water from the disc and discard the water.

4. To the tube (with the disc) add:
   a. 5 µl of the 10X BLUE buffer
   b. 1 µl *forensic*GEM
   c. 44 µl Nuclease-free water

5. Vortex each sample for 5 seconds. Pulse spin in a mini centrifuge to remove droplets from the lid and walls of the tube.

6. In a thermocycler, incubate:
   a. 75°C for 5 min
   b. 95°C for 5 min

7. Pipette the solution to a new tube. The paper can be discarded.

8. The sample is in the solution (DO NOT DISCARD) and ready for analysis. Vortex and pulse spin before using. Typically, 2-5 µl should be used in PCR.

DNA extraction from whole blood (liquid) (forensicGEM Universal kit, RED+ buffer)

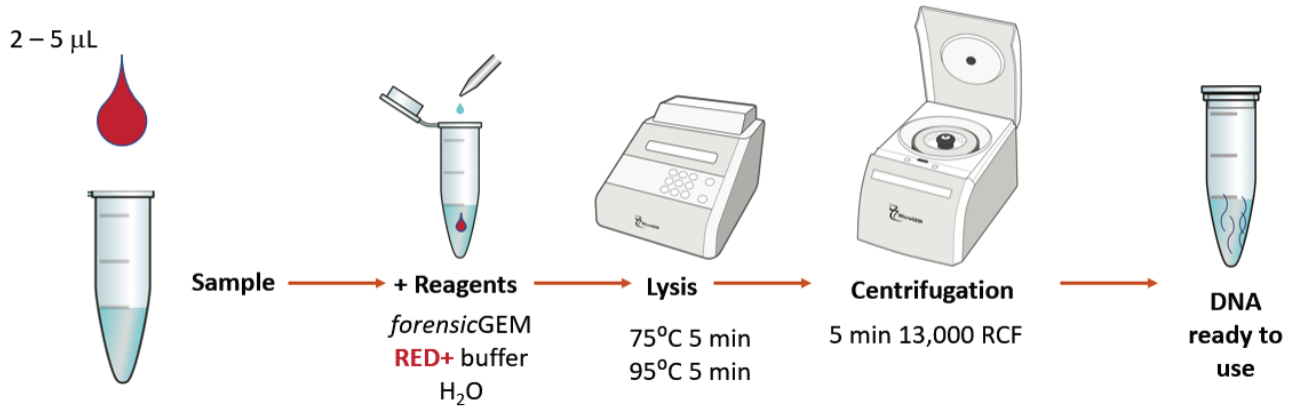


Figure 6. Workflow for DNA extraction from liquid blood.

MicroGEM's RED+ buffer is a proprietary formulation that precipitates PCR inhibitors. The solid material should not be disturbed when removing the supernatant (Step 6).

Yields will vary depending on the White Blood Cell (WBC) count of the sample.

You should be aware heme coloration carries through to the DNA extract leaving the sample slightly pink. This does not cause inhibition of downstream PCR or qPCR.

1. Pipette 2-5 µl of blood into a thin-walled PCR tube or a 96-well plate.

2. In a thin-walled PCR tube add:
   a. 10 µl of the 10X RED+ buffer
   b. 1 µl *forensic*GEM
   c. 87-84 µl Nuclease-free water (Total volume 100 µl)

3. Vortex to mix.

4. In a thermocycler, incubate:
   a. 75°C for 5 min
   b. 95°C for 5 min

5. Centrifuge for 5 minutes at maximum speed (~13,000 RCF). (Typically, 5 minutes at 13,000 relative centrifugal force [RCF] is sufficient to give a well-packed pellet. Longer spins should be used for lower RCF centrifugations. For example, a typical 96-well plate, swing out rotor rated at 3,000 RCF should be spun 10 minutes. Centrifugation should be performed immediately after extraction).

6. Pipette the supernatant to a new tube without disturbing the pellet. (As the forensicGEM buffer is a proprietary formulation that precipitates PCR inhibitors make sure to not disturb the solid material when removing the supernatant).

7. The DNA is in the solution (DO NOT DISCARD) and ready for analysis. Vortex and pulse spin before using.
***NOTE:** there may be some discoloration (yellow-pink-brown) of the solution due to heme from the blood. Yields of ~0.5ng/µl can be expected from fresh blood (2-5 µl).

DNA extraction from whole blood (storage cards) (forensicGEM Universal kit, RED+ buffer)

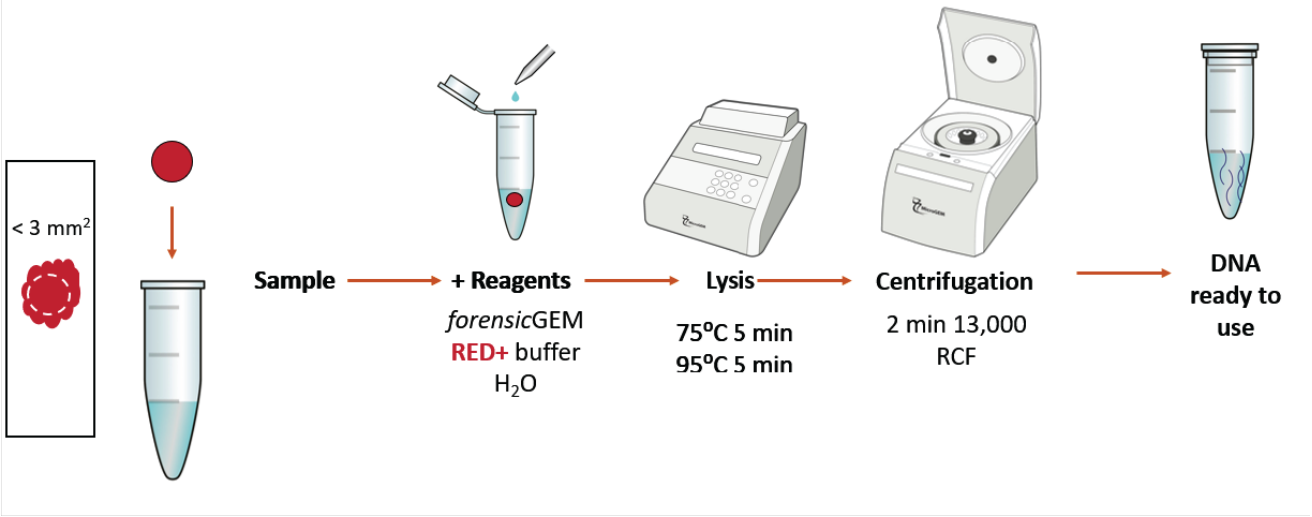


Figure 7. Workflow for DNA extraction from blood on storage cards.

Depending on the storage card, it is typical that the preservatives in the card are inhibitory to Taq DNA polymerase. To remove these inhibitors, a prewash is recommended prior to DNA extraction.

1. Remove one 3 mm² disc from the card-stored blood sample and place into a thin-walled PCR tube or a 96-well plate. For the best results, punch in the center of the area where the blood was applied.

2. Wash the disc in 100 µl of Nuclease-free water by incubating at room temperature for 15 minutes. Aspirate the water from the disc(s) and discard. This prewash step is intended to remove any inhibitors that could be inhibitory to Taq DNA polymerases. Place disc into a thin-walled PCR tube.

3. In a thin-walled PCR tube add:

a. 5 µl of the 10X RED+ buffer

b. 1 µl forensicGEM

c. 44 µl Nuclease-free water

4. In a thermocycler, incubate:

a. 75°C for 5 min

b. 95°C for 5 min

5. Centrifuge for 2 minutes at maximum speed (~13,000 RCF). (Typically, 2 minutes is sufficient. Longer spins should be used for lower RCF centrifugations. For example, a typical 96-well plate, swing out rotor rated at 3,000 RCF should be spun for 10 minutes. Centrifugation should be performed immediately after extraction).

6. Pipette the supernatant to a new tube without disturbing the pellet/disc. (As the forensicGEM buffer is a proprietary formulation that precipitates PCR inhibitors make sure to not disturb the solid material when removing the supernatant). The disc can be discarded.

7. The DNA is in the solution (DO NOT DISCARD) and ready for analysis. Vortex and pulse spin before using.

***NOTE:** there may be some discoloration (yellow-pink-brown) of the solution due to heme from the blood.

DNA extraction from human tissue (forensicGEM Universal kit, ORANGE+ buffer)

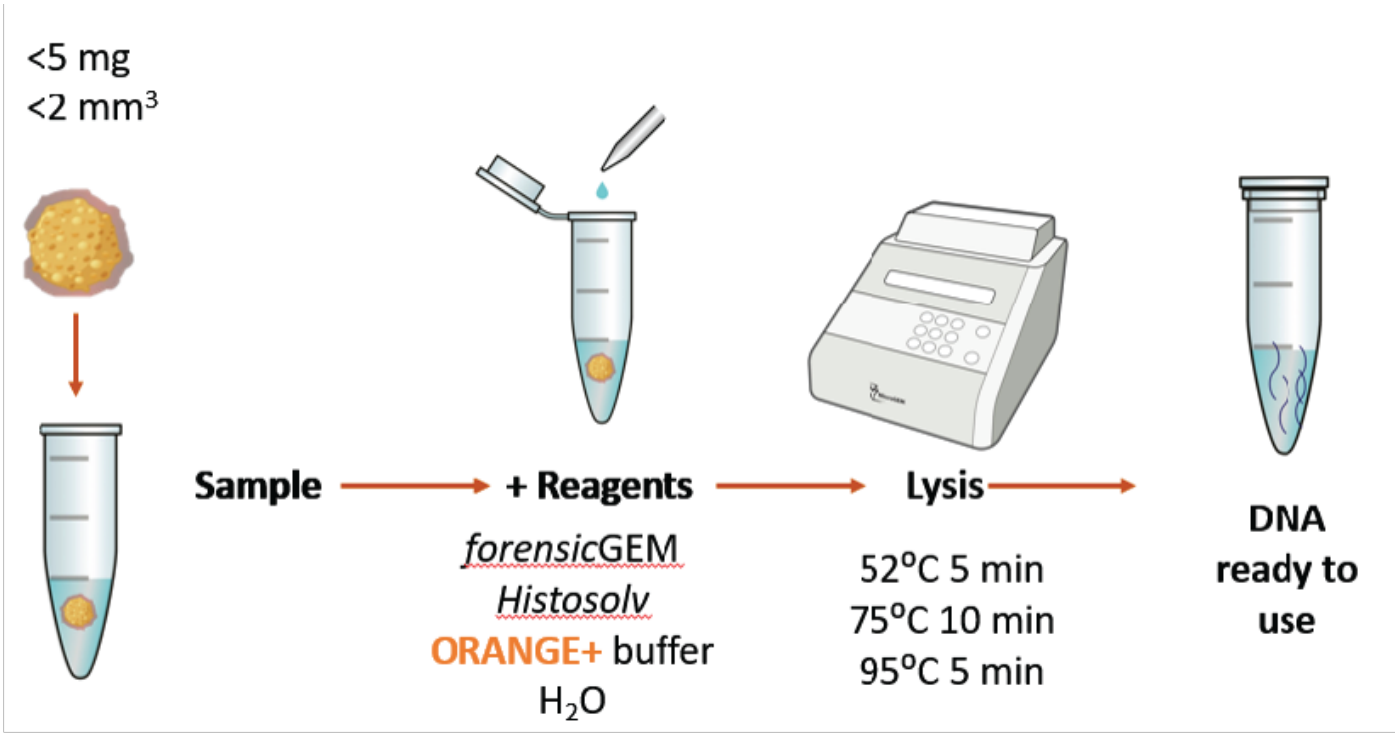


Figure 8. Workflow for DNA extraction from tissue samples.

1. Cut the tissue into pieces of approximately 1-2 mm³. With hair follicles, use 1-3 hairs. Cut off the shaft 4 mm above the follicle. Place sample into a thin-walled PCR tube or a 96-well plate.

2. In a thin-walled PCR tube add:
   a. 10 µl of the 10X ORANGE+ buffer
   b. 1 µl *forensic*GEM
   c. 10 µl Histosolv
   d. 79 µl Nuclease-free water
3. Mash the sample with a pipette tip and disperse by vortexing.

4. In a thermocycler, incubate:
   a. 52°C for 5 min
   b. 75°C for 10 min
   c. 95°C for 5 min

5. Aspirate the extract away from the residual material.

6. The DNA is in the solution (DO NOT DISCARD) and ready for analysis. Vortex and pulse spin before using.

DNA extraction from bloodstains (forensicGEM Universal, RED+ buffer)

The same method used for extraction for blood on storage cards can be used with bloodstains. Methods will vary depending on the sample type.

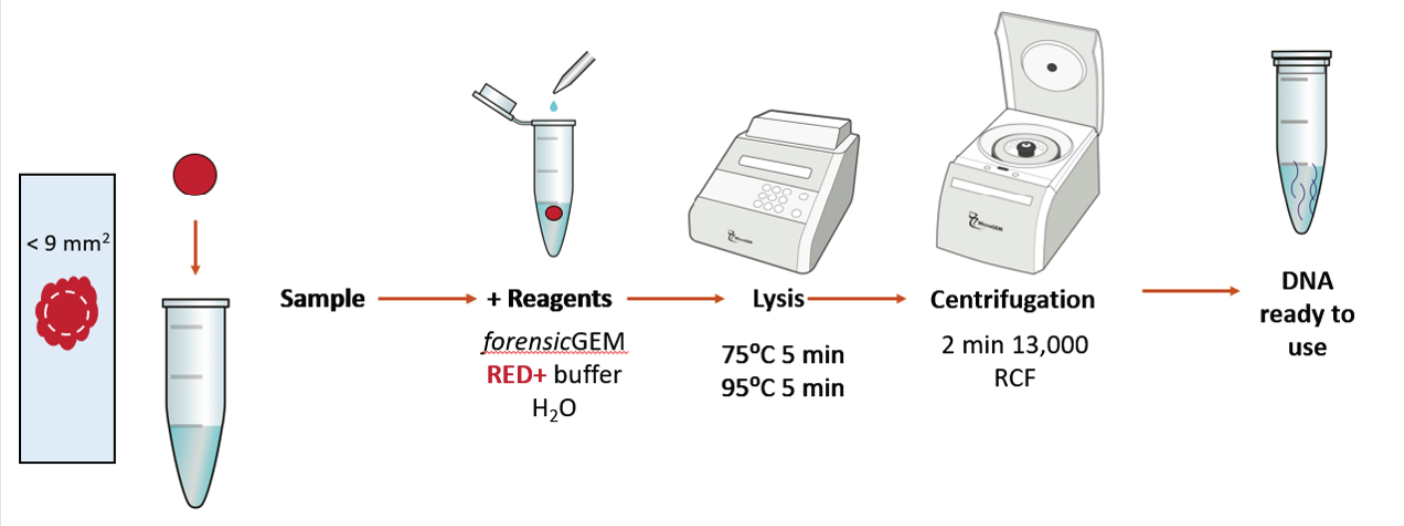


Figure 9. Workflow for DNA extraction from bloodstains.

1. Take bloodstained sample no larger than 9 mm². Place into a thin-walled PCR tube or a 96-well plate.

2. To the PCR tube add:
   a. 5 µl of the 10X RED+ buffer
   b. 1 µl *forensic*GEM
   c. 44 µl Nuclease-free water

3. In a thermocycler, incubate:
   a. 75°C for 5 min
   b. 95°C for 5 min

4. Centrifuge for 2 minutes at maximum speed (~13,000 RCF). (Typically, 2 minutes at 13,000 relative centrifugal force (RCF) is sufficient. Longer spins should be used for lower RCF centrifugations. For example, a typical 96-well plate, swing out rotor rated at 3,000 RCF should be spun 10 minutes. Centrifugation should be performed immediately after extraction).

5. Pipette the supernatant to a new tube without disturbing the pellet/sample. (As the forensicGEM buffer is a proprietary formulation that precipitates PCR inhibitors make sure to not disturb the solid material when removing the supernatant).

6. The DNA is in the solution (DO NOT DISCARD) and ready for analysis. Vortex and pulse spin before using.

***NOTE:** there may be some discoloration (yellow-pink-brown) of the solution due to heme from the blood.

*forensic*GEM SPERM PROTOCOL

DNA extraction from semen (liquid, swabs, stains) (forensicGEM Sperm kit, ORANGE+ buffer)

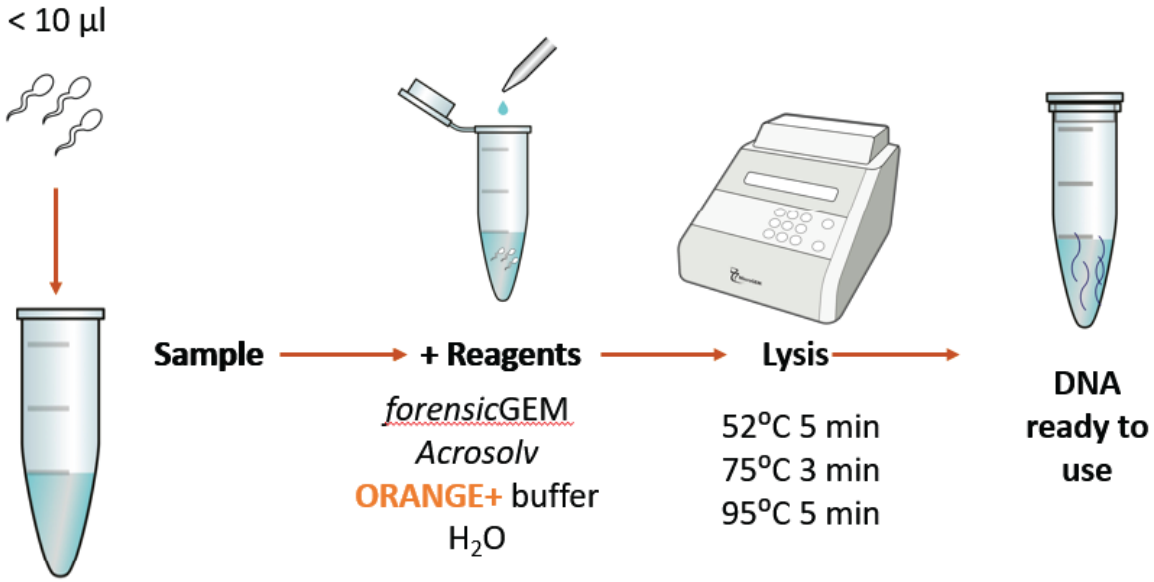


Figure 10. Workflow for DNA extraction from semen.

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 ¼ of the swab directly to the extraction solution. Stained fabric can be swabbed, or small portions added directly to the extraction solution.

1. Place a (<9 mm²) sample into a thin-walled PCR tube or a 96-well plate.

2. Into a thin-walled PCR tube, add:
    a. 10 µl of the 10X ORANGE+ buffer
    b. 2 µl *forensic*GEM Sperm
    c. 10 µl Acrosolv
    d. Nuclease-free water to 50 µl

3. Mix the sample by vortexing.

4. In a thermocycler, incubate:
    a. 52°C for 5 min
    b. 75°C for 3 min
    c. 95°C for 5 min

5. Aspirate the extract away from any residual material.

6. The DNA is in the solution (DO NOT DISCARD) and ready for analysis. Vortex and pulse spin before using.

TROUBLESHOOTING

This troubleshooting guide may be helpful in solving any problems that may arise, we have also included some protocol modifications to aid in extraction from atypical samples not specifically addressed in the handbook, for further help please reach out to techsupport@exymesplc.com.

Troubleshooting post extraction application issues:

	Issue	Possible Causes	Potential Solutions
1	Inaccurate nucleic acid quantification	Exymes' s extracts are not compatible with Nanodrop, or UV based quantification methods.	As an alternative, use fluorometric quantification methods, such as Quant˜s, Qubit, PicoGreen-DNA etc., or quantitative PCR (qPCR).
2	Observed PCR or qPCR inhibition	Co-extraction of inhibitors from too much starting material or extracting longer than recommended. Too much DNA template added into PCR mix. PCR/qPCR primers are not working effectively.	Perform a new PCR with a lower amount of DNA (1-10 ng) as a template. 1 ng of DNA is usually sufficient for most PCR/qPCR applications.
			Add BSA to your PCR mix (5-20%, or 1 µl of a 10 mg/ml solution in 25 µl reaction).
			Carry out a serial dilution of the extracted RNA or DNA to allow for less DNA to be used as a template for the PCR.
			Ensure the problem is due to inhibition and not due to a low concentration of DNA (this can be done by looking at the slope and endpoint of a qPCR plot compared to a positive control). Running PCR controls will inform you as to whether the PCR mix and/or primers are working as intended.
			Centrifuge extract and aspirate supernatant into a new tube (this step is not normally needed) but if there is obvious solid material in extract this can reduce PCR efficiency.
			Carry out a downstream purification step such as using magnetic beads or silica columns to further purify the DNA extract.

	Issue	Possible Causes	Potential Solutions
3	Observed low yield of extracted nucleic acids	Incorrect storage of starting material.	DNA yield is dependent on type, size, age and storage of starting material, lower yields can be expected from older improperly stored samples.
		Buffer incorrectly prepared.	Make sure to vortex the buffer completely before use, if you see any precipitates, incubate at 37°C until dissolved, then use.
		Sample/extraction buffer not mixed properly.	Mix your sample with the extraction buffer by pulse vortexing for 15 seconds before heating.
		Improper storage of extraction reagents.	Ensure all of your sample is immersed in the extraction buffer. This may require increasing the volume of the extraction buffer.
		Incompatible quantification method used.	Increase the amount of sample input and/or the volume of the extraction reagents.
			For larger samples be sure to vortex and/or homogenize the sample prior to extraction.
			Ensure you are using a compatible quantification method such as fluorometric dyes, qPCR. (See #1 of the troubleshooting section above).
4	Extracted DNA is sheared	MicroGEM' s lysis method should result in minimal fragmentation of the DNA due to a gentle lysis method and the lack of bead or column-based purification.	DNA yield is dependent on type, size, age and storage of starting material, lower yields can be expected from older improperly stored samples.
		The likely cause of fragmentation is due to improper sample handling such as the sample being too old and/or the sample undergoing multiple freeze/thaw cycles.	Try to use fresher samples.
			Avoid repeated freeze/thaw cycles of your starting material.
5	Observed interference with downstream enzymes	MicroGEM' s proteinase insufficiently inactivated.	Ensure the extraction mix, not just the heating apparatus, reaches 95°C for specified time in protocol (5 min).

	Issue	Possible Causes	Potential Solutions
6	Observed low yield of extracted nucleic acids from blood samples	Incorrect storage of starting material. Buffer incorrectly prepared. Sample and buffer not mixed properly.	Make sure to closely follow the MicroGEM extraction protocols
		EDTA present in blood sample.	If chelating agents like EDTA are present, add 10 mM CaCl° to the extraction (2 µl in a 100 µl reaction volume).
		Low white blood count (WBC) in blood sample.	Ensure you are using a compatible quantification method such as fluorometric dyes, qPCR. (See #1 of the troubleshooting section above).

Troubleshooting for atypical sample input extraction issues:

	Sample Type	Sample Issue	Potential Solutions
1	Dried tissue	Dried tissue/sample absorbs too much of the extraction mix and results in an inadequate extract volume.	Rehydrate the tissue in water or extraction buffer (without enzyme) at 4°C overnight prior to extraction to limit the amount of extraction mix absorption.
2	Tough tissue	Tougher tissue types (i.e., lung, kidney) give lower DNA yield.	Increase volume of enzyme in the extraction mix (e.g., 2X).
			Increase the 75°C incubation time to extract more DNA. A 30-minute 75°C incubation should be sufficient.
3	Hair follicles	Unsure how many hair follicles should be used for extraction.	1 - 3 hair follicles should be sufficient to extract enough DNA extraction for PCR. We do not recommend the addition of more than 10 hair follicles in the extraction mix.

	Sample Type	Sample Issue	Potential Solutions
4	Sperm	Sperm samples give lower than expected yield. Possible insufficient sperm cell lysis.	Increase the Acrosolv incubation (52°C) time to lyse more sperm heads.
			Increase 75°C incubation can also help to extract more DNA from the lysed sperm cells.
5	Tissue extraction	Unsure how much tissue to use as starting material in the extraction.	We recommend <5 mg or <2 mm² of solid tissue (such as mouse tails).
			It is generally better to work with a smaller amount of solid tissue to limit the co-extract of too many PCR inhibitors.
6	Blood	Are all blood storage (anticoagulants) compatible with MicroGEM extractions?	Heparin (green top tubes) and sodium citrate (light blue top tubes) work without modification to the protocol. EDTA is a chelating agent, so if using EDTA (purple top tubes) add 2 µL CaCl2 [10 mM CaCl2] per 100 µl extraction.
7	Swabs	Some swabs have binding agents or fine particles that can affect PCR.	Test swab for inhibition by washing in water and adding to PCR control.
		Buccal cells sediment rapidly in wash solution.	Mix suspension immediately before transferring to extraction buffer.
8	Tough tissue type (animal)	Low yield from certain animal tissue types (pancreas, spleen, lung).	Add 0.5 non-ionic detergent such as Triton X-100.

ORDERING / DISTRIBUTION

Ordering

Information regarding ordering is provided below. To contact our Commercial Team, please email commercial@exymesplc.com

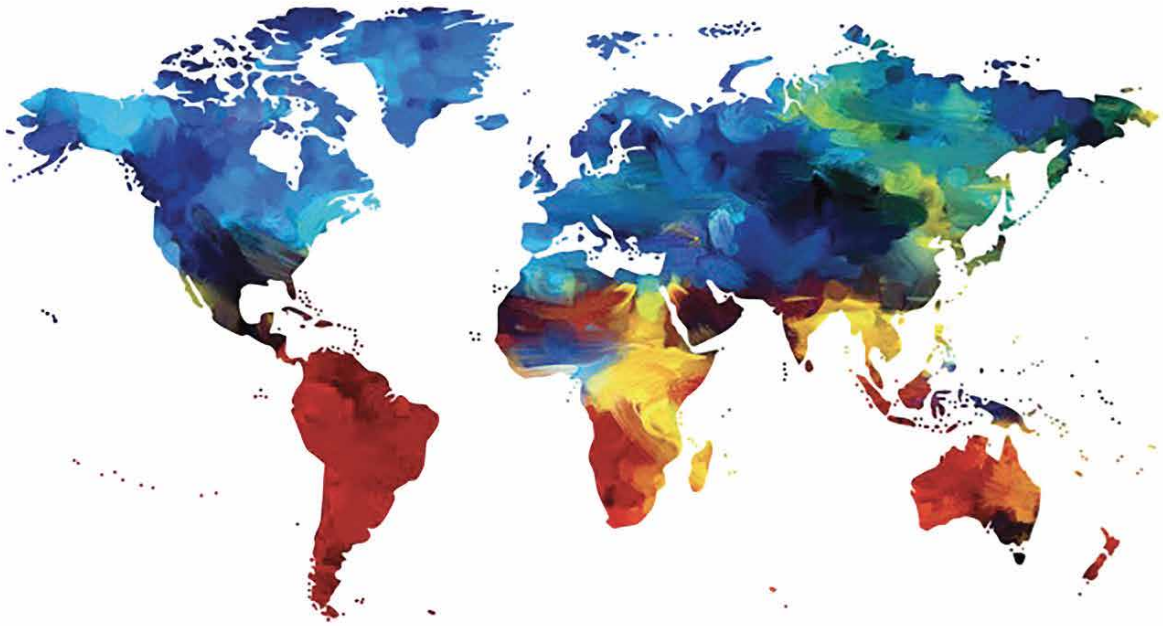
Kit Type	Kit Reaction Number	Product Code
forensicGEM Universal 100 reactions	forensicGEM 10X BLUE buffer 10X ORANGE+ buffer 10X RED+ buffer Histosolv	FUN0100
forensicGEM Universal 500 reactions	forensicGEM 10X BLUE buffer 10X ORANGE+ buffer 10X RED+ buffer Histosolv	FUN0500
forensicGEM Universal 1000 reactions	forensicGEM 10X BLUE buffer 10X ORANGE+ buffer 10X RED+ buffer Histosolv	FUN1000
forensicGEM Sperm 100 reactions	forensicGEM 10X ORANGE+ buffer Acrosolv	FSC0100
forensicGEM Sperm 500 reactions	forensicGEM 10X ORANGE+ buffer Acrosolv	FSC0500
forensicGEM Sperm 1000 reactions	forensicGEM 10X ORANGE+ buffer Acrosolv	FSC1000

Distribution

Information about Exymes' s distributors can be found on the Exymes Website: www.exymesplc.com

References

(1) Hudson, B. C.; Cox, J. O.; Seashols-Williams, S. J.; Dawson Cruz, T. The Effects of Dithiothreitol (DTT) on Fluorescent QPCR Dyes. J. Forensic Sci. 2021, 66 (2), 700–708. https://doi.org/10.1111/1556-4029.14637



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At Exymes, our goal is to provide ultra fast nucleic extraction enabling a broader spectrum of users to both employ and benefit from molecular techniques. The first step is the simplification of sample preparation. Our temperature-driven, single-tube process simplifies and reduces the number of steps for traditional nucleic acid extraction, resulting in high-quality extracts with reduced contamination and high yields - all in minutes, not hours.

Committed to minimal packaging, reduced plastic use and a sustainable world

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