*RNA*GEM Handbook

*RNA*GEM | *RNA*GEM V

MicroGEM

C0094 RevA

CONTENTS

KIT CONTENTS AND STORAGE

MicroGEM 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 overage while preparing Master Mix for extractions ensuring customers get the full number of reactions out of their kits.

*RNA*GEM Kit Contents:

Kit contains: *RNA*GEM, 10X **BLUE** buffer, 10X *DNase buffer,* 10X TE buffer, *DNase I*

Table 1. Kit components for *forensic*GEM Universal.

Component	Volumes				Temperature (shipping)	Temperature (storage)
	50 rxn	100 rxn	500 rxn	1,000 rxn		
Catalogue no.	PUN0050	PUN0100	PUN0500	PUN01000		
*RNA*GEM*	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
DNase buffer 10X	1 ml	1 ml	5 ml	10 ml	RT	4°C
10X TE buffer	1 ml	1 ml	5 ml	10 ml	RT	4°C
DNase I	50 rxn	100 rxn	500 rxn	1,000 rxn	RT	-20°C
DNase I (rehydrated)**	Prepared by customer					-20°C

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

****NOTE:** Once *DNase I* has been rehydrated, it is stable for 7 months at -20°C. If you do not plan to use all the *DNase I* immediately, it is recommended that you aliquot *DNase I* into smaller volumes and store at -20°C.

*RNA*GEM Kit Contents:

Kit contains: *RNA*GEM, 10X **BLUE** buffer

Table 2. Kit components for *forensic*GEM Sperm.

Component	Volumes		Temp. (shipping)	Temp. (storage)
	50 rxn	1,000 rxn		
Catalogue no.	PUN0050	PUN01000		
*RNA*GEM*	50 µl	1000 µl	RT	-20°C
10X **BLUE** buffer	1 ml	10 ml	RT	4°C

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

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. *RNA*GEM must be stored at -20°C, potentially in aliquots to reduce the number of freeze/thaw cycles. *DNase I* must be stored at -20°C prior to being rehydrated. Once hydrated, *DNase I* 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 Use Limitations

MicroGEM 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 MicroGEM 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

MicroGEM guarantees the performance of all products in the manner described in our product literature. The purchaser should determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, MicroGEM 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 MicroGEM product does not meet your expectations, simply contact Technical Service (techsupport@microgembio.com) or distributor. We will credit your account or exchange the product – as you wish.

A copy of MicroGEM 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@microgembio.com) or your distributor.

Technical Assistance

At MicroGEM, 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 MicroGEM products. If you have any questions or experience any difficulties regarding *RNA*GEM Kits or MicroGEM products in general, please do not hesitate to contact us, techsupport@microgembio.com.
MicroGEM 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 MicroGEM. We therefore encourage you to contact us if you have any suggestions about product performance, publications or new applications and techniques.
For technical assistance and more information, please contact our Technical Support team at techsupport@microgembio.com.

Quality Control

MicroGEM 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:

*RNA*GEM

https://microgembio.com/wp-content/uploads/2019/03/RNAGEM_SDS_20190730.pdf

*RNA*GEM V

https://microgembio.com/wp-content/uploads/2021/05/SDS002_V1_20200327_RNAGEM-V-1.pdf

Product Principle/Product Overview

MicroGEM'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, becoming active at 75°C, lysing cells, and removing nucleoproteins from the DNA and RNA, 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 by traditional extraction methods (i.e., silica columns, magnetic beads, or organic extractions) reducing time and increasing sample processing. The *RNA*GEM and *RNA*GEM-V kits both extract total nucleic acids suitable for applications such as PCR, RT-PCR and qPCR or RT-qPCR. The MicroGEM *RNA*GEM kits are designed for purification of RNA and/or DNA from a variety of sample types. This includes but is not limited to saliva, cells and insects, using the *RNA*GEM kit (RTP). For swabs or saliva samples where both DNA and RNA from viruses are of interest, *RNA*GEM-V is recommended.

Procedure Overview (*RNA*GEM)

RNA Extraction (NO *DNase I*)

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

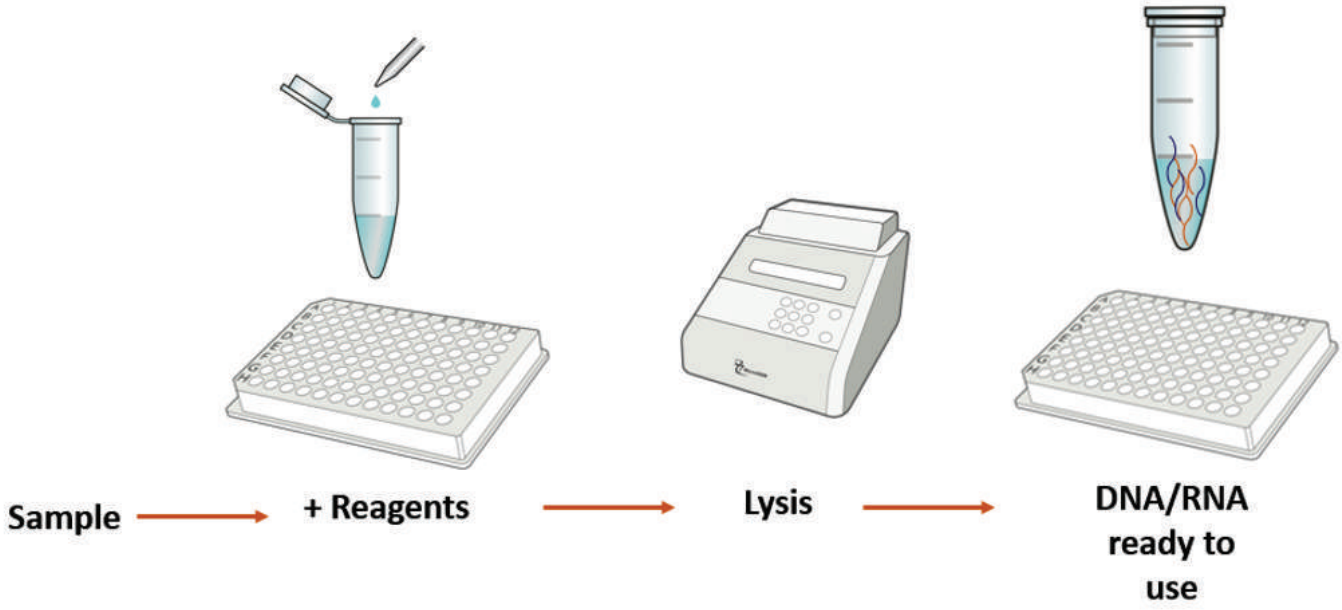


Figure 1.

Simple workflow for RNA extraction utilizing Temperature Driven Extraction (TDE). No DNase treatment is included. Simple temperature changes activate the enzyme to extract total nucleic acids, free of proteins. This reaction can be carried out in a single tube or scaled up to a 96-well plate and can be programmed for a thermocycler or robots and liquid handlers.

Procedure Overview (*RNA*GEM)
RNA Extraction (NO *DNase I*)

Workflow for extraction from sample to RNA in < 30 min 1 to 96 samples.

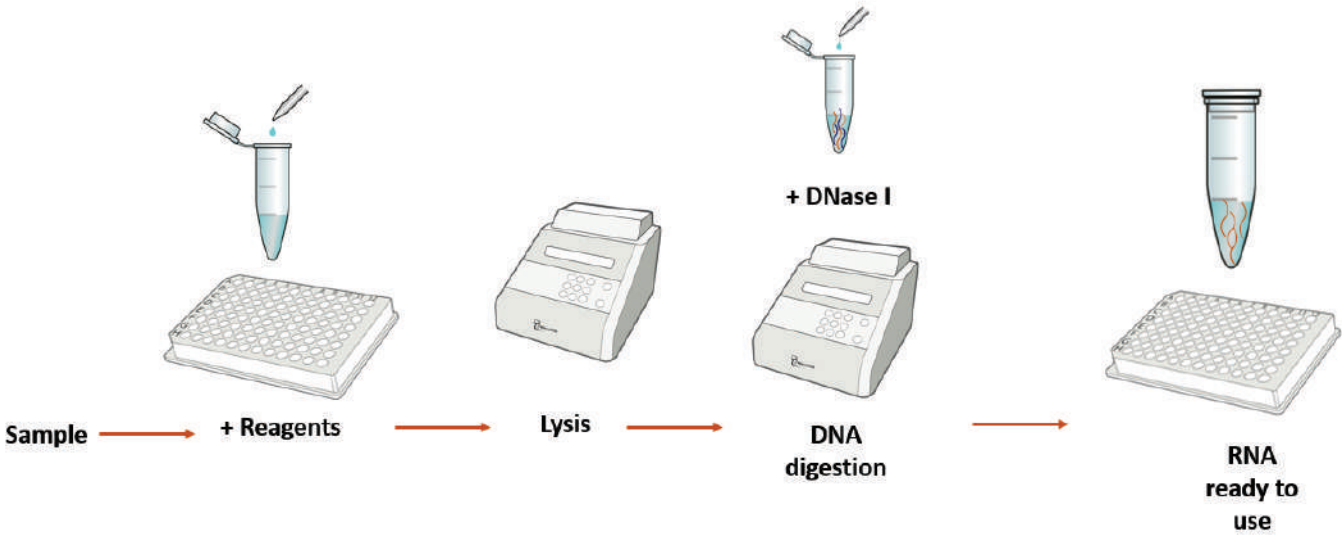


Figure 2.
Simple workflow for RNA extraction utilizing Temperature Driven Extraction (TDE), Optional DNase I treatment is included. Simple temperature changes activate the enzyme to extract total RNA, free of proteins. This reaction can be carried out in a single tube or scaled up to a 96-well plate and can be programmed for a thermocycler or robots and liquid handlers.

Equipment and Reagents to be Supplied by User

For all protocols:

Equipment

Vortexer
Pipettes
Thermocycler or heat block

Consumables

Pipette tips
Nuclease-free water

TECHNICAL TIPS

- The method, enzyme formulation, and buffer have been carefully optimized for extracting nucleic acids. Using the enzyme with other methods or buffers is not recommended. If you wish to modify the method in any way, please email: techsupport@microgembio.com.
- 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).
- For accurate quantification, an RT-qPCR is recommended.
  Additional quantification information can be found here (https://microgembio.com/wp-content/uploads/2021/11/Quantification-App-Note-compressed_1.pdf)
- 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 storage of extracted RNA add 1/10th of the total extract volume of the 10X TE buffer provided and store at -20°C or below.

PRE-EXTRACTION STEPS

Pre-Extraction (RNAGEM): Resuspend the DNase I

DNase I is delivered as a dry powder. Before it is ready to be used, the powder should be dissolved in 1X DNase buffer (provided as a 10X solution). Different kit sizes contain tubes with different amounts of enzyme (ensure you follow the directions on the *DNase I* label in your kit). Be sure to add the correct amount of water (see the table below). MicroGEM supplies extra enzyme to compensate for minor pipetting errors.

1. Centrifuge the *DNase I* tube for 1 minute at 10,000 RCF. This will settle the powder in the bottom of the tube. The tube may appear empty.

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

Table 3. Table showing rehydration volumes for *DNase I*

Component	Product Code	10X DNase buffer	Nuclease-free water	Total volume
50 rxn	RTP0050	11 µl	99 µl	11 µl
100 rxn	RTP0100	22 µl	198 µl	220 µl
500 rxn	RTP0500	110 µl	990 µl	1,100 µl
1000 rxn	RTP1000	220 ml	1,980 µl	2,200 µl

3. Vortex and store at -20°C. The concentration of the DNAse solution will be 1 U/µl.

Prepare Master Mix

In order to ensure that the yields are uniform amongst samples, it is recommended that an extraction Master Mix is prepared prior to performing extractions. The Master Mix can then be either added to sample in tubes or put into tubes prior to adding sample. We recommend using the *RNA*GEM reagents within one hour of preparation. For longer periods, reagents should be frozen.

An example of a Master Mix preparation is shown below:

Table 4. Example calculation for preparing Master Mix for 10 cell pellet samples.

Component	Volume per single extraction (µl)	Number of Reactions +1 (overage)	Total volume added (µl)
10X **BLUE** buffer	5	11	55
Nuclease-free water	44	11	484
*RNA*GEM	1	11	11

RNA Extraction from Cells (suspension, adherent, cell pellets, RNAlater™)

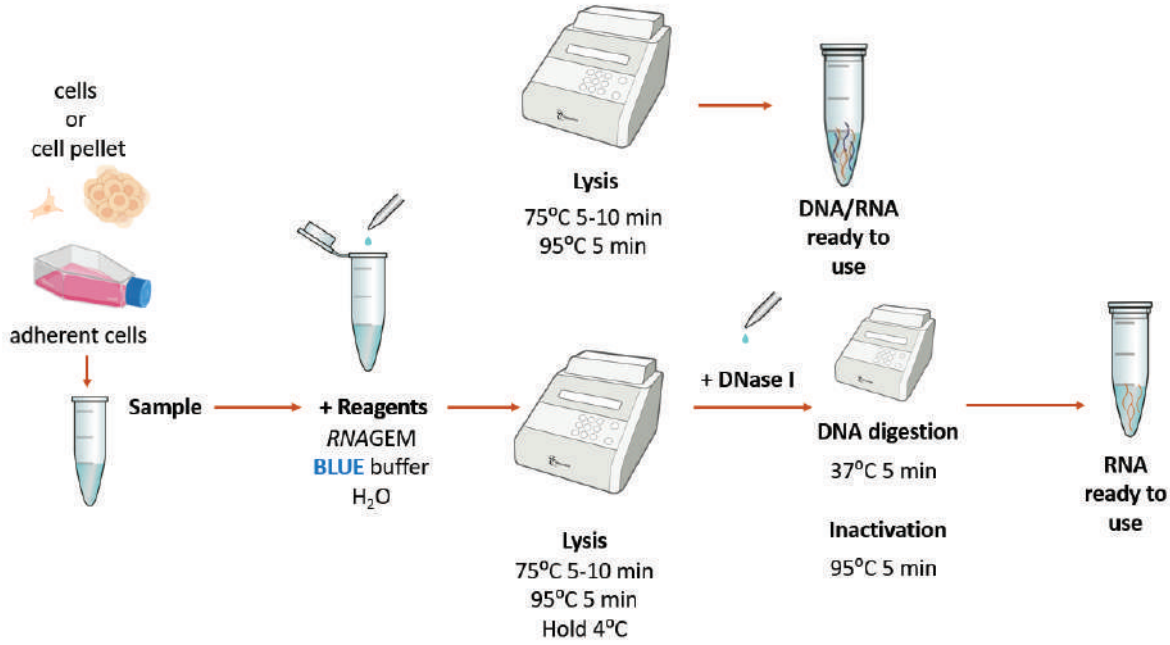


Figure 3. Workflow for RNA extraction from cells.

Sample Preparation

Sample Prep - Cells in suspension:

1. Centrifuge the suspension at 200 RCF for 5 minutes.
2. Remove all the liquid.
3. Resuspend the pellet in *RNA*GEM extraction solution.

Sample Prep - Adherent cells:

If the cells are in flasks, dislodge cells by preferred method (Trypsin or cell scraper) and centrifuge suspension at 200 RCF for 5 minutes. Otherwise, the *RNA*GEM extraction solution can be added directly to the cell monolayer.

*RNA*GEM reagents are sensitive to EDTA and other chelating agents. If cells are presented in an EDTA-containing solution, they should be centrifuged at 200 RCF and washed in 1X **BLUE** buffer before extraction.

1. Centrifuge the suspension at 200 RCF for 5 minutes.
2. Remove all the liquid.
3. Resuspend the pellet in *RNA*GEM extraction solution.

Sample Preparation - Cell pellets:

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

Sample Preparation - FACS and LCM:

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

Sample Preparation - RNAlater™:

RNAlater™ is a somewhat viscous solution and contains inhibitory compounds that need to be removed before extraction (this is required for most extraction processes).

1. Centrifuge suspension at 3,000 RCF for 5 minutes.
2. Remove all of the liquid (a quick spin on the benchtop microcentrifuge can help gather the last few drops).
3. Resuspend the pellet in the *RNA*GEM extraction solution.

Extraction Procedure

1. Mix your sample with the *RNA*GEM reagents by using the volumes in the following table.

Table 5. Volume of reagents to use in extraction buffer preparation based upon cell numbers.

Number of cells	Volume of RNAGEM	Volume of BLUE buffer (1/10 of total volume)	Volume of Nuclease-free water	Total extraction volume
50,000 - 500,000	1 µl	5 - 10 µl	44 - 89 µl	50 - 100 µl
5,000 - 50,000	1 µl	2 - 5 µl	17 - 44 µl	20 - 50 µl
100 - 5,000	0.5 µl	0.5 - 2 µl	4 - 17.5 µl	5 - 20 µl
1 - 500	0.2 µl	0.1 - 1.5 µl	0.7 - 13.3 µl	1 - 15 µl

2. Vortex and incubate at:
    a. 75°C
        i. >50,000 cells 10 min
        ii. <50,000 cells 5 min

    b. 95°C for 5 min

(DO NOT CARRY OUT THIS STEP if you are planning to continue on to do a DNA digestion)

3. Your extract containing DNA and RNA (total nucleic acids) is now ready to use. Vortex and spin before using.

DNA Digestion (optional)

4. To 50 µl of extract, add:
    a. 5 µl 10X *DNase* buffer
    b. 2 µl *DNase I*

5. Vortex and incubate:
    a. 37°C for 5 min
    b. 95°C for 5 min

6. Your RNA extract is now ready to use. Vortex and spin before using.

RNA Extraction from swabs stored in transport media

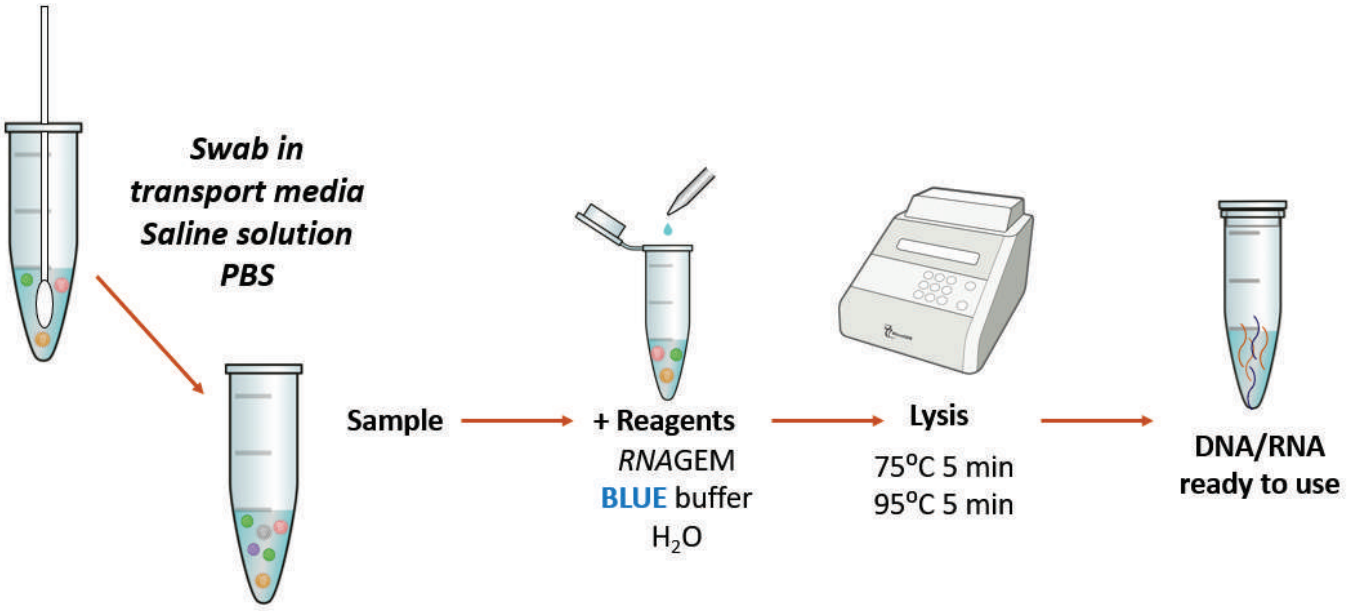


Figure 4. Workflow for DNA/RNA extraction from swabs.

IMPORTANT:
*RNA*GEM V has been shown to be compatible with swabs collected in 2 ml saline solution (0.9% NaCl) and PBS. Lower volumes can lead to inhibition of the RT-qPCR, producing delay in Ct values. In addition, *RNA*GEM V is also compatible with some UTM media. Incompatible UTM media may lead to Ct delays.

1. Vortex the swab in the transport media.
2. Pipette 89 µl of the transport buffer into a thin-walled PCR tube or 96-well plate.
3. Add the following reagents to the tube (the total volume should be 100 µl):

    a. 10 µl of the 10X **BLUE** buffer
    b. 1 µl *RNA*GEM

4. In a thermocycler, incubate

    a. 75°C for 10 min
    b. 95°C for 5 min

4. This solution now contans DNA and RNA (Total Nucleic Acid) ready for RT-PCR and RT-qPCR. Vortex and spin before using.

RNA Extraction from virus in saliva

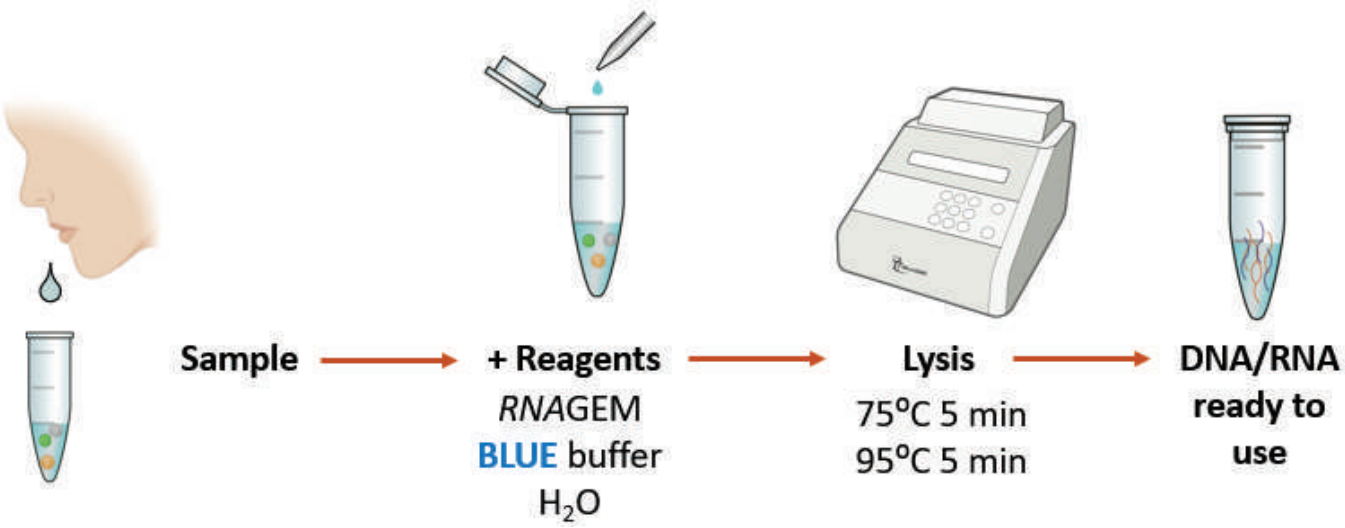


Figure 5. Workflow for DNA/RNA extraction from saliva.

1. Pipette 45 µl of the saliva sample and 44 µl of Nuclease-free water into a thin-walled PCR tube or 96-well plate.

2. Add the following reagents to the tube (the total volume should be 100 ml):

    a. 10 µl of the 10X **BLUE** buffer
    b. 1 µl *RNA*GEM

3. In a thermocycler, incubate

    a. 75°C for 10 min
    b. 95°C for 5 min

4. This solution now contans DNA and RNA (Total Nucleic Acid) ready for RT-PCR and RT-qPCR. Vortex and 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 samples not specifically addressed in the handbook, for further help please reach out to techsupport@microgembio.com.

Troubleshooting post extraction application issues:

	Issue	Possible Causes	Potential Solutions
1	Inaccurate nucleic acid quantification	IMicroGEM' s extracts are not compatible with Nanodrop, or UV based quantification methods.	As an alternative, use fluorometric quantification methods, such as Quantrs, 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 RNA 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 NA to be used as a template for the PCR/RT-PCR.
			Ensure the problem is due to inhibition and not due to a low concentration of RNA or DNA (this can be done by looking at the slope and endpoint of a RT-qPCR/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 transfer 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 RNA or DNA extract.

	Issue	Possible Causes	Potential Solutions
3	Observed RNA/DNA degredation storage	MicroGEM' s storage recommendations were not followed correctly.	If you do not intend to analyze the extracted RNA/DNA immediately, then add to the extract 10% of the 10X TE buffer (100 mM Tris [pH 7.5], 10 mM EDTA).
4	Incomplete DNA digestion in RNA extractions	*DNase* not rehydrated in *DNase* buffer.	Make sure the extract is cool before adding the *DNase I* and *DNase* buffer.
		Extract still "hot" when adding *DNase I* reagents.	Make sure to properly read instruction for *DNase* step.
5	Concentration of extract too low for downstream application	MicroGEM' s temperature driven extraction method does not include concentration steps in the procedure. The concentration of the extract is dependent on the quality of the sample and the extraction volume used.	Scale up/down the protocol (use more or less than the suggested volume of extraction reagents) according to your concentration requirements. Lower total volume of extraction reagents will likely lead to an increased final extraction concentration. Alternatively, you could use a downstream concentration method such as magnetic beads or silica columns to concentrate your extract.

	Issue	Possible Causes	Potential Solutions
6	Observed low yield of extracted nucleic acids	Incorrect storage of starting material. Buffer incorrectly prepared. Sample/extraction buffer not mixed properly. Improper storage of extraction reagents. Incompatible quantification method used.	RNA and/or DNA yield is dependent on type, size, age and storage of starting material, lower yields can be expected from older improperly stored samples.
			Make sure to vortex the buffer completely before use, if you see any precipitates, incubate at 37°C until dissolved, then use.
			Mix your sample with the extraction buffer by pulse vortexing for 15 sec before heating.
			Ensure all of your sample is immersed in the extraction buffer. This may require increasing the volume of the extraction buffer.
			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).
7	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.	
		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.

	Issue	Possible Causes	Potential Solutions
8	Observed interference of 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).
9	Observed background signal when using universal primers (16S rRNA gene primers)	Interference from PCR reagents	Please refer to literature for best practices to minimize interference from contaminating DNA in PCR reagents.

Troubleshooting for atypical sample input extraction issues:

	Issue	Possible Causes	Potential Solutions
1	Insects	Can RNA be extracted from insects?	Homogenize insect, 1µl *RNA*GEM, 5 µl **BLUE** buffer, 44 µl water 75°C 15 min, 95°C 5 min.
		Would like intact/non-homogenized insect	Increase 75°C incubation time to 120 min.
2	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.
3	Transport media	Transport media can affect RT-qPCR results.	*RNA*GEM V is compatible with swabs collected in saline (0.9% NaCl) and PBS. However, some transport medias (UTMs) are incompatible and may lead to Ct value delays in RT-qPCR.

ORDERING INFO

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

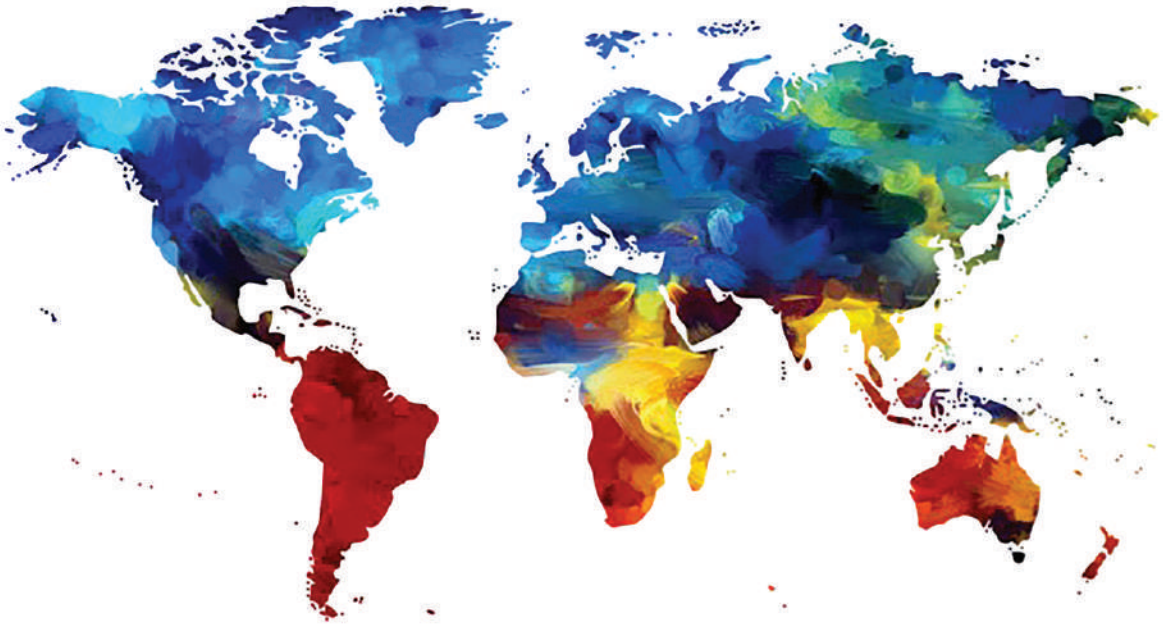
Product	Kit Contents	Product Code
*RNA*GEM 50 reactions	*RNA*GEM 10X **BLUE** buffer *DNase I* 10X *DNase* buffer 10X TE buffer	RTP0050
*RNA*GEM 100 reactions	*RNA*GEM 10X **BLUE** buffer *DNase I* 10X *DNase* buffer 10X TE buffer	RTP0100
*RNA*GEM 500 reactions	*RNA*GEM 10X **BLUE** buffer *DNase I* 10X *DNase* buffer 10X TE buffer	RTP0500
*RNA*GEM 1000 reactions	*RNA*GEM 10X **BLUE** buffer *DNase I* 10X *DNase* buffer 10X TE buffer	RTP1000
*RNA*GEM V 50 reactions	*RNA*GEM 10X **BLUE** buffer	RTV0050
*RNA*GEM V 1000 reactions	*RNA*GEM 10X **BLUE** buffer	RTV1000

Distribution

Information about MicroGEM' s distributors can be found on the MicroGEM Website: www.microgembio.com

References

(1) Amouroux, P.; Crochard, D.; Correa, M.; Groussier, G.; Kreiter, P.; Roman, C.; Guerrieri, E.; Garonna, A.; Malausa, T.; Zaviezo, T. Natural Enemies of Armored Scales (Hemiptera: Diaspididae) and Soft Scales (Hemiptera: Coccidae) in Chile: Molecular and Morphological Identification. PLOS ONE 2019, 14 (3), e0205475. https://doi.org/10.1371/journal.pone.0205475.



techsupport@microgembio.com | microgembio.com

At MicroGEM, our goal is to democratize molecular biology, 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.

MicroGEM

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