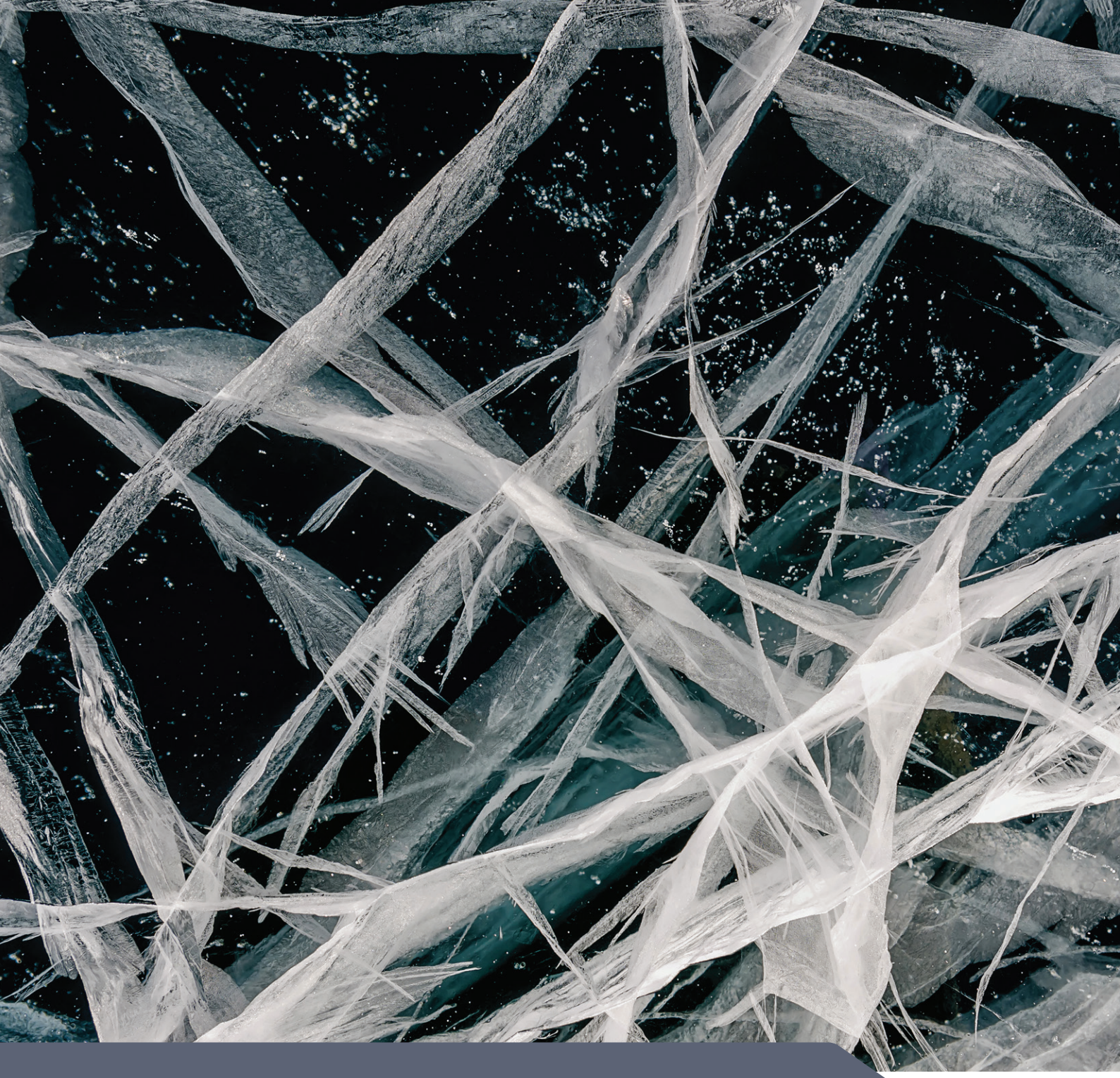


*RNA*GEM Handbook

*RNA*GEM

Exymes

C0082 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. Exymes 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 *RNA*GEM.

Component	Volumes				Temperature (shipping)	Temperature (storage)
	50 rxn	100 rxn	500 rxn	1,000 rxn		
Catalogue no.	PUN0050	PUN0100	PUN0500	PUN01000		
RNAGEM*	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, RNAGEM 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.

Storage

Once received Exymes 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. Histosolv and lysozyme 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 and Enhancer 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 prepGEM 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

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective eyewear. For more information, please consult the appropriate safety data sheets (SDSs). 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, 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 RNAGEM and RNAGEM-V kits both extract total nucleic acids suitable for applications such as PCR, RT-PCR and qPCR or RT-qPCR. The Exymes RNAGEM 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 RNAGEM kit (RTP). For swabs or saliva samples where both DNA and RNA from viruses are of interest, RNAGEM-V is recommended.

PROCEDURE OVERVIEW

RNA Extraction (NO DNase I)

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

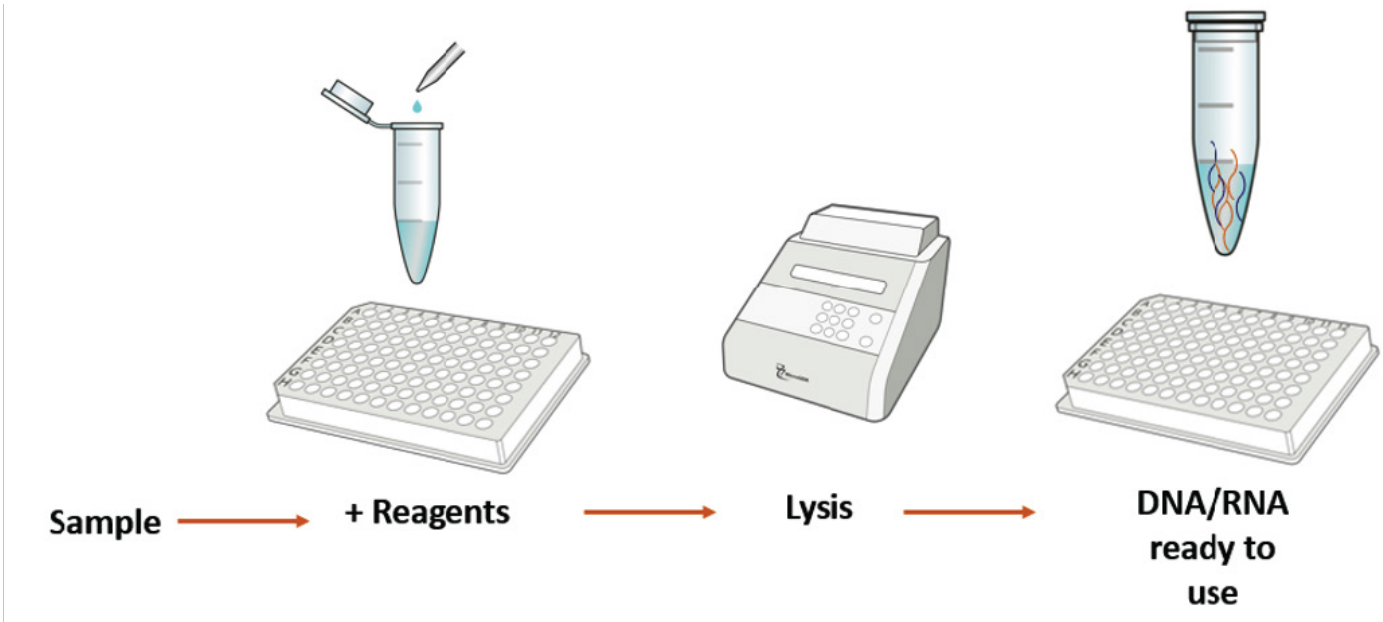


Figure 1. Simple workflow for RNA extraction utilizing Temperature Driven Extraction (TDE). No DNase treatment is included. Simple temperature changes activate the enzyme to extract total nucleic acids, free of proteins. This reaction can be carried out in a single tube or scaled up to a 96-well plate and can be programmed for a thermocycler or robots and liquid handlers.

RNA Extraction with DNase I Treatment

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

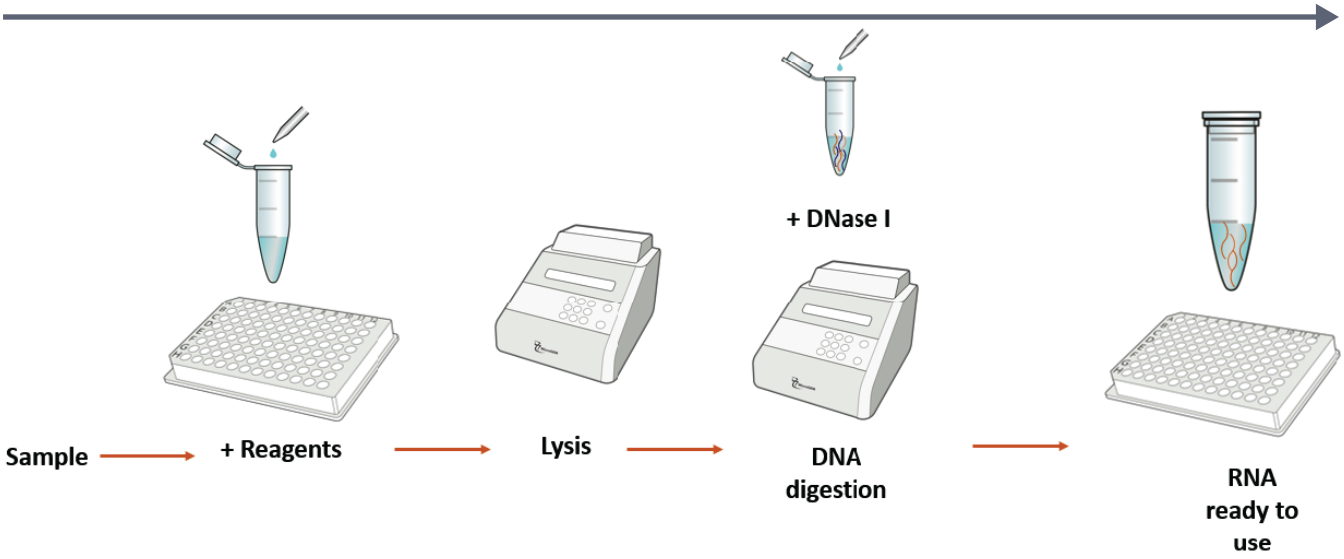


Figure 2. Simple workflow for RNA extraction utilizing Temperature Driven Extraction (TDE), Optional DNase I treatment is included. Simple temperature changes activate the enzyme to extract total RNA, free of proteins. This reaction can be carried out in a single tube or scaled up to a 96-well plate 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 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.
- 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 in the Quantification Application Note 016.
- 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). Exymes 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 RNAGEM 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
RNAGEM	1	11	11

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

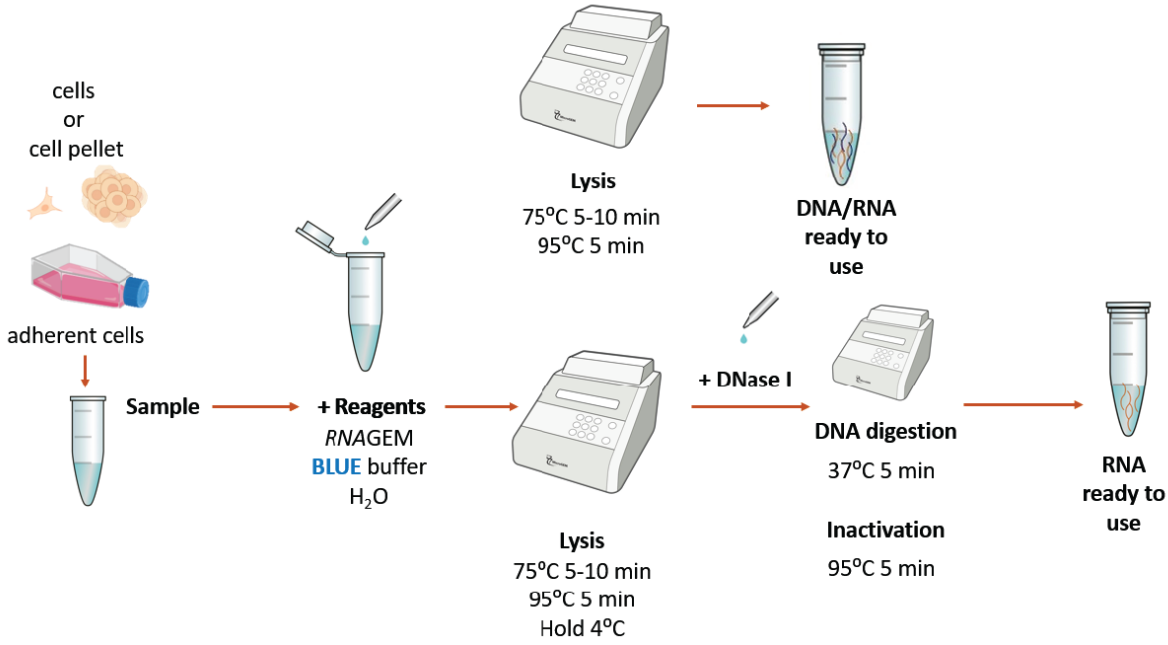


Figure 3. Workflow for RNA extraction from cells.

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 RNAGEM 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 RNAGEM extraction solution can be added directly to the cell monolayer.

RNAGEM 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 RNAGEM extraction solution.

Sample Preparation - Cell pellets:

Up to 5 x 10^6 cells can be extracted using the recommended method. Linear extraction efficiency is best achieved within the range of <10 cells to approximately 10^6 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 RNAGEM 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 RNAGEM extraction solution.

Extraction Procedure

1. Mix your sample with the RNAGEM 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

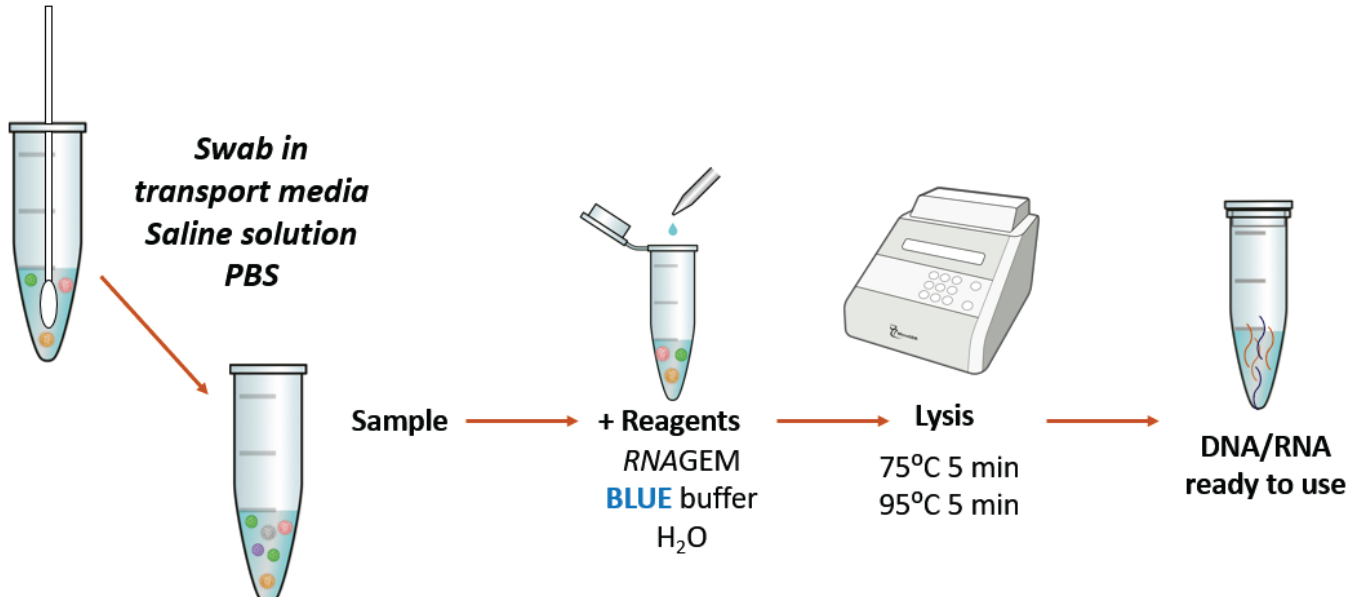


Figure 4. Workflow for DNA/RNA extraction from swabs.

IMPORTANT:

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

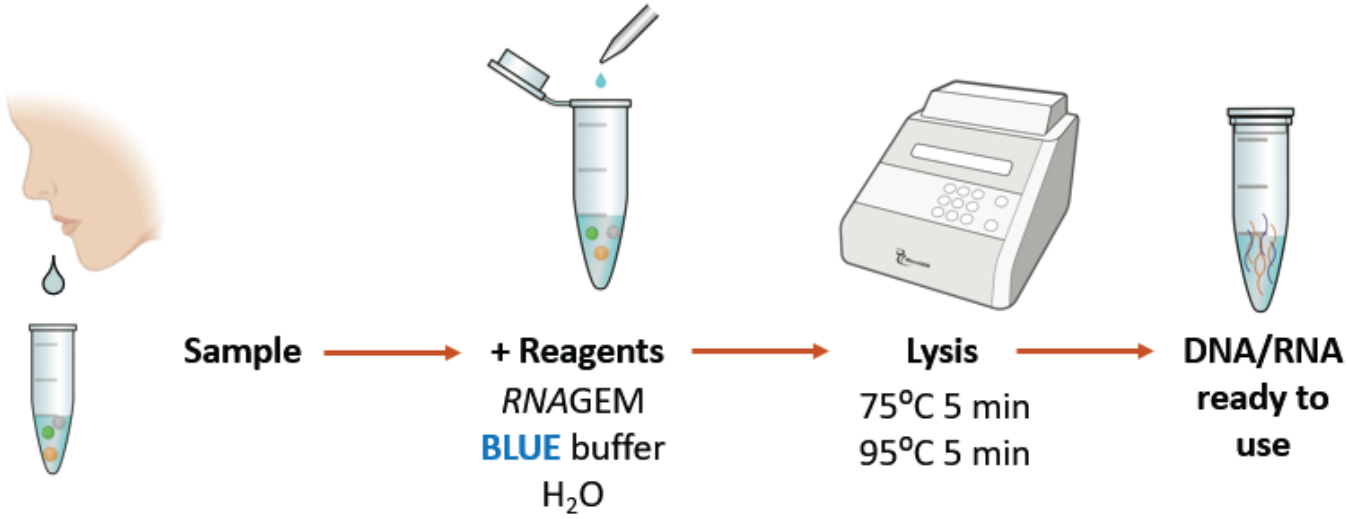


Figure 5. Workflow for DNA/RNA extraction from saliva.

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 RNAGEM

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@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 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	Exymes' 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	Exymes' 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.
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).

	Issue	Possible Causes	Potential Solutions
7	Extracted DNA is sheared	Exymes' 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.
8	Observed interference of downstream enzymes	Exymes'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 RNAGEM, 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.	RNAGEM 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@exymesplc.com

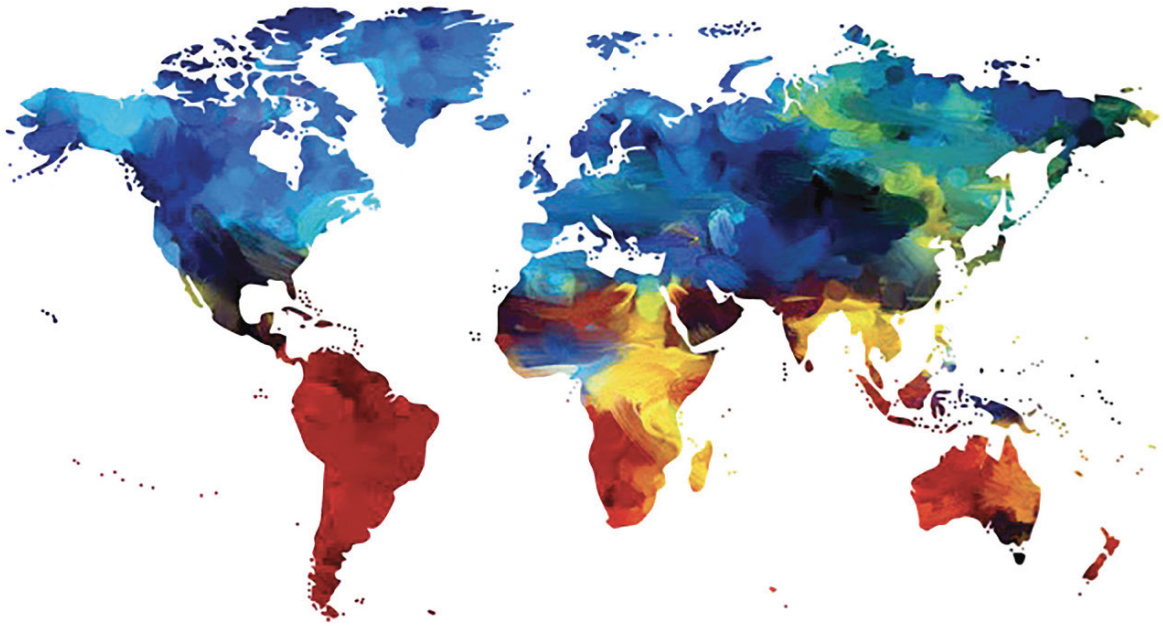
Product	Kit Contents	Product Code
RNAGEM 100 reactions	RNAGEM 10X BLUE buffer DNase I 10X DNase buffer 10X TE buffer	RTP0100
RNAGEM 500 reactions	RNAGEM 10X BLUE buffer DNase I 10X DNase buffer 10X TE buffer	RTP0500
RNAGEM 1000 reactions	RNAGEM 10X BLUE buffer DNase I 10X DNase buffer 10X TE buffer	RTP1000

Distribution

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



info@exymesplc.com | www.exymesplc.com

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

Exymes