eXymes Quick-Start Guide

DNA Extraction Using

*RNA*GEM™ Universal

Ultra Fast Nucleic Acid Extraction

Find more information at
www.exymesplc.com

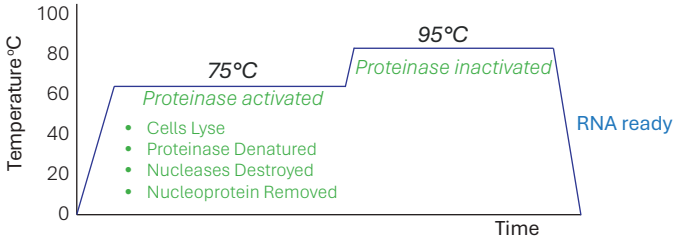
or email
info@exymesplc.com

*RNA*GEM™

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

Principles of the Extraction

eXymes extraction products use a unique mixture of thermophilic and mesophilic enzymes. Protocols involve a 75°C step to activate eXymes's thermophilic proteinase to lyse cells, denature nucleases, and strip DNA of nucleosomes. A final 95°C step deactivates the thermophilic proteinase resulting in analytics-ready RNA.



Kit Components and Reagent Storage

*RNA*GEM reagents are delivered at room temperature but on arrival should be stored at 4°C. After tubes have been opened, the *RNA*GEM enzyme should be placed at -20°C. Once DNase 1 has been rehydrated, it is stable for 7 months at -20°C. If you do not plan to use all of the DNase 1 immediately, it is recommended that you aliquot DNase 1 into smaller volumes and store at -20°C immediately after rehydration. The buffers can remain at 4°C for convenience.

Reagent QC

ZyGEM extraction products use a unique thermophilic proteinase. Extractions are performed at 75°C and the proteinase is inactivated at 95°C. A thermal cycler is used for this process.

General Instructions, Precautions and Technical Tips

- The method, enzyme formulation and buffer have been carefully optimised for extracting intact RNA. Using the enzyme with other methods or buffers is not recommended.
- If you need to modift the method in any way, please email: info@exymesplc.com for advice.
- Absorbance 260/280 nm is an ineffective quantitation method with *RNA*GEM-prepared nucleic acids. For accurate quantification we recommend RT-qPCR and normalisation to genomic DNA using a reference gene, or fluorometric based assays.
- As with any method of RNA preparation, the best results are obtained when samples are handled on ice in an RNAse-free environment and using certified RNAse-free tubes and reagents.
- For long-term storage, RNA should be stored at -80°C.
- Alternatively, RNA in TE buffer can be precipitated using NH_4OAc/ ethanol (0.1 volumes of 5 M NH_4OAc, and 2.5 volumes of 100% ethanol) and stored at -20°C or below
- *RNA*GEM is sensitive to EDTA and other chelating agents. If cells are presented in EDTA-containing solutions, they should be centrifuged at 2000 x g and washed in 1x BLUE buffer before use.
- Cells can be collected directly in the extraction reagent mastermix or the reagents added directly to a capillary from LCM. If cells are collected in a different buffer, it may be necessary to add 1/10th volume of the eXymes buffer after collection. We recommend using eXymes reagents within one hour of preparation. For longer periods, reagents should be frozen.

Resuspending the DNAse I

DNAse I is delivered as a lyophilised powder. Before it is ready to use, the powder should be dissolved in 1x DNAse I Reaction Buffer (provided as a 10x solution). Different kit sizes contain tubes with different amounts of enzyme (READ THE LABEL). Be sure to add the correct amount of buffer (see the table on the next page).

1. Centrifuge the DNAse I tube for 1 minute at 10,000 x g. This will settle the powder to the bottom of the tube.
2. In a clean environment, open the tube and add:

DNase Rxn Size	10x DNase Buffer	RNase-Free Water	Total Volume for Reconstitution
50	11	99	110
100	22	198	220
500	110	990	1100
1000	2x 500 Rxns	2x 500 Rxns	2x 500 Rxns

(eXymes supplies extra activity to cater for pipetting error)

3. Vortex and store at -20°C. The concentration of this solution will be 1 Unit per µl.

Unit Definition: 1 Unit causes an increase in absorbance at 260nm of 0.001 per minute per ml at 25°C when acting upon highly polymerised DNA at pH 5.0. 0.005 Kunitz unit digests 1 µg of lambda DNA in 10 minutes at 37°C in 50 mM Tris, 1 mM Mg2+, pH 7.8 in a 50 µl reaction.

Sample Preparation and Extraction Procedures

*RNA*GEM is a kit for extracting total nucleic acids from mammalian tissue culture and is optimised for producing RNA. The method lyses cells and digests proteins and ribonucleases. Extracted RNA can be used for RT-PCR and RT-qPCR.

All manipulations should be performed in an RNAse-free environment or a PCR hood.

Use only certified RNAse-free tubes and reagents. *RNA*GEM gives linear yields for 10 to approx 10^5 cells and is ideal for single-cell work. For low numbers of cells we recommend reducing the extraction volume. The minimum volume possible will depend on evaporation with the equipment you are using. The recommended amounts of *RNA*GEM to use for different extraction volumes are below. Use 1/10th volume of 10x BLUE buffer.

Extraction Volume	Cell Numbers	Volume of RNAGEM
50 - 100 µl	50,000 - 500,000	1 µl
20 - 50 µl	5,000 - 50,000	1 µl
5 - 20 µl	100 - 5,000	0.5 µl
1 - 15 µl	1 - 500	0.2 µl

Sample handling will vary with different sample types. An outline of some suggested procedures is provided on the back page of this document. More information is available at *www.exymesplc.com*.

Handling Different Culture Types

Cells in Suspension

1. Centrifuge the suspension at 200 x g for 5 mins.
2. Remove all of the liquid.
3. Resuspend the pellet in *RNA*GEM extraction reagents.

Adherent Cells

If the cells are in flasks, dislodge cells by preferred method (Trypsin or cell scraper) and centrifuge suspension at 200 x g for 5 mins. Otherwise, the eXymes reagents can be added directly to the adhered layer.

1. Remove all of the liquid.
2. Add *RNA*GEM extraction reagents.

Cells Stored in RNAlater™

1. Centrifuge suspension at 3,000 x g for 5 mins.
2. Remove all of the liquid (a quick spin on a bench centrifuge can help to gather the last few drops).
3. Resuspend the pellet in *RNA*GEM extraction reagents.

Cell pellets

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

Procedure

1. *Add:*
   Cell suspension or pellet
   5 µl 10x BLUE Buffer
   1 µl *RNA*GEM
   Water to a final volume of 50 µl

2. *Vortex and incubate:*

   75°C for 5 min (< 50,000 cells) or 10 min (> 50,000 cells)
   95°C 5 mins (skip if DNA digestion is required)
   4°C HOLD

   (A thermal cycler should be used for this step.)

DNase Treatment (IF REQUIRED)

(Scale for different extraction volumes)

1. To the extract add:
   5 µl of the 10x DNAse buffer
   2 µl DNAse I
2. Vortex and incubate:
   37°C for 5 minutes
   95°C for 5 minutes
   4°C HOLD

3. Add 1/10th volume of 10x TE Buffer (provided) and store at -20°C or below.

Troubleshooting

For troubleshooting, please see our guide “eXymes Method Optimization and Troubleshooting” in the resources area of the eXymes website.

Additional Information

For publications and app notes on your specific sample type, please visit our website.

More information can be found at :
www.exymesplc.com

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