MicroGEM Quick-Start Guide

RNA Extraction Using

*RNA*GEM

Find more information at
www.microgembio.com

or email
info@microgembio.com

QSG_010_190531_RNAGEM

When your kit arrives

All of the components in the RNAGEM kit are stable at room temperature and will arrive at your laboratory at ambient temperature. However, once you have opened any tube or resuspended your DNAse I in buffer, the components should be stored as follows:

10x Buffers	4°C
RNAGEM™ and DNAse I	-20°C

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 below).

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
50 Rxn	11 µl	99 µl
100 Rxn	25 µl	225 µl
200 Rxn	44 µl	376 µl
500 Rxn	105 µl	945 µl

(MicroGEM 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 handling

RNAGEM 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.*

RNAGEM 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 RNAGEM to use for different extraction volumes are below. Use 1/10th volume of 10 X **BLUE** buffer.

Extraction Volume	Cell numbers	Volume of RNAGEM
50 - 100 µl	50,000 - 500,000	1 µl
20 - 50 µl	5000 - 50,000	1 µl
5 - 20 µl	100 - 5000	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.microgembio.com.

NOTE: RNAGEM is sensitive to EDTA and other chelating agents. If cells are presented in EDTA-containing solutions, they should be centrifuged at 200 x g and washed in 1X **BLUE** buffer before use.

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 MicroGEM 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.

FACS and LCM

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 MicroGEM buffer after collection. We recommend using MicroGEM reagents within one hour of preparation. For longer periods, reagents should be frozen.

Procedure

(See notes on scaling for different volumes)

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
   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
   75°C for 5 minutes
   4°C HOLD

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

Technical tips and sample management

- 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 modify the method in any way, please email: support@microgembio.com.

- 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.

More information is available on our website at www.microgembio.com