MicroGEM

*RNA*GEM (RTP)

QUICK START CARD

Component	Shipped at	Storage Temperature
*RNA*GEM	RT	-20°C
10X BLUE buffer	RT	4°C
DNase I	RT (shipped as a powder)	-20°C
10X DNase buffer	RT	4°C
10X TE buffer	RT	4°C

For the Safety Data Sheet, please scan this QR Code.

For the most up-to-date information, troubleshooting guides, and detailed instructions based on your sample type, please see the *RNA*GEM Handbook using this QR code before starting.

For technical assistance:
techsupport@microgembio.com

Instructions before starting:

Pick your sample type

The processing of the sample will vary depending on sample type. The following protocols has been validated with cells, please contact us for guidance on how to extract from other sample types

DNA Digestion

The *RNA*GEM kit will extract total nucleic acids. If you require just RNA, you can carry out a DNA digestion using the DNase I and DNase buffer provided in the kit

Scalable Protocols

The *RNA*GEM protocols are scalable based on the number of cells in the sample. You can use the table below to determine the total volume of reagents and component breakdown needed for your sample.

Cell Numbers	Volume of *RNA*GEM	Volume of 10X BLUE Buffer	Total Extraction Volume
50,000-500,000	1 µl	5-10 µl	50-100 µl
5,000-50,000	1 µl	2-5 µl	20-50 µl
100-5,000	0.5 µl	0.5-2 µl	5-20 µl
1-500	0.2 µl	0.1-1.5 µl	1-15 µl

Resuspending 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 RCF. 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	Nuclease-free Water	Total Volume for Reconstitution
50	11 µl	99 µl	110 µl
100	22 µl	198 µl	220 µl
500	110 µl	990 µl	1100 µl
1000	2 x 500 Rxns	2 x 500 Rxns	2 x 500 Rxns

3. Vortex and store at -20°C. The concentration of this solution will be 1 unit µl.
(MicroGEM supplies extra amount to accomodate for pipetting error)

Quantification

MicroGEM extracts are not compatible with UV-based quantification methods. For information on how to accurately quantify extracts, please look at the *RNA*GEM Handbook.

Handling Different Culture Types

Cells in Suspension

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

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 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 as reported below.

Cells Stored in RNAlater ™

1. Centrifuge the suspension at 3000 RCF 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 solution as reported below.

Optional: Wash the pellet with 1 ml ice-cold PBS, centrifuge at 400 RCF for 5 mins and discard the supernatant.

Cell Extraction

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.

RNA extraction protocol (without DNase)

1. Prepare

Make up the extraction mixture (volumes shown below are for a 50 µl reaction).

Cell suspension or pellet
5 µl 10X **BLUE** buffer
1 µl *RNA*GEM
Water to final volume of 50 µl

2. Extract

Vortex, spin down and incubate

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

3. Ready

The solution contains total nucleic acid.

For long term storage, add 1X TE buffer and store at -20°C

DNA Digestion (if required)

1. Add

Ensuring the extract has cooled to 4˚C, add:

2 µl DNase I
5 µl 10X DNase buffer

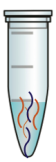
2. Incubate

Vortex, spin down and incubate

37°C for 5 min
95°C for 5 min
4°C hold

3. Ready

The solution contains RNA.



For long term storage, add 1X TE buffer and store at -20°C

MicroGEM