ZyGEM Quick-Start Guide

RNA Extraction Using *RNA*GEM Tissue PLUS

rapid, enabling solutions for Biotechnology

See information at www.zygem.com
or email support@zygem.com

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*RNA*GEM Tissue *PLUS*

Sample preparation and handling

*RNA*GEM Tissue *PLUS* is a kit for extracting total nucleic acids from mammalian tissue culture. The kit contains DNAse I for the removal of DNA and is designed for producing RNA. The method lyses cells and digests proteins and ribonucleases. The RNA produced by this kit can be used for RT-PCR and RT-qPCR

The reagents are stable at room temperature but on arrival should be stored at 4°C. After opening the tubes and re-suspending the DNAse I powder, the tubes should be stored at -20°C.

- Resuspend the DNAse 1 powder as specified on the seperate instruction sheet.
- Use only certified RNAse-free tubes and reagents.

*RNA*GEM gives linear yields for 10 to ~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 1/10^{th} volume of 10x SILVER buffer.

Extraction Volume	Cell numbers	Volume of *RNA*GEM™
50 µl	50,000 - 500,000	1 µl
20 - 50 µl	5000 - 50,000	1 µl
5 - 20 µl	500 - 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.zygem.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 ZyGEM 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 SILVER buffer and an appropriate quantity added to the extraction.

FACS and LCM

Cells can be collected directly in the extraction reagent master-mix 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 ZyGEM buffer after collection. We recommend using ZyGEM reagents within one hour of preparation. For longer periods, reagents should be frozen.

*RNA*GEM 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 SILVER buffer before use.

Extraction *(50 µl reaction - see notes on scaling)*

1. Add:
   Cell suspension or pellet (see notes on scaling)
   5 µl 10x Buffer **SILVER**
   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 *(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 1/10^{th} volume of 10x TE Buffer (provided). Store at -20°C.

Sample management and storage.

- As with any of method 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 5M NH_4OAc, and 2.5 volumes 100% ethanol) and stored at -20°C or below.
- Absorbance 260/280 nm is an ineffective quantitation method with *RNA*GEM-prepared nucleic acids. See website for details.

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