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rapid, enabling solutions
for Biotechnology



RNA Extraction Using RNA GEM™ Tissue

Zygem Quick-Start Guide

RNA GEM™ Tissue

Sample preparation and handling

RNA GEM™ Tissue 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.

The reagents are stable at room temperature but on arrival should be stored at 4°C. After tubes have been opened, the enzyme should be placed at -20°C to safeguard against contamination.

- 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 SILVER buffer.

Extraction Volume	Cell numbers	Volume of RNA GEM™
50 - 100 µl	50,000 - 100,000	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.zygem.com.

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And then fold here

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

1. Dislodge cells by preferred method (Trypsin or cell scraper).
2. Centrifuge suspension at 200 x g for 5 mins.
3. Remove all of the liquid (a quick spin on a bench centrifuge can help to gather the last few drops).
4. Resuspend the pellet in 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 5×10^5 cells can be extracted using the recommended method. Linear extraction efficiency is best achieved within the range of $<10^5$ cells to approximately 10^6 . 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 mastermix or the reagents added directly to a capillary from LCM. 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	
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.
3. Add 1/10th volume of 10x TE Buffer (provided) and store at -20°C or below.

Technical tips and sample management

- The method, the 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@zygem.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.
- 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 100% ethanol) and stored at -20°C or below.

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(RNAGEM™ Tissue With DNase I)

RNA Extraction Using RNAGEM™ Tissue PLUS

ZyGEM Quick-Start Guide

RNAGEM™ Tissue PLUS

Sample preparation and handling

RNAGEM™ 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 in the Enzyme Resuspension buffer, the tubes should be placed at -20°C.

- Resuspend the DNase I powder in 1X DNase Reaction Buffer. Use the amount specified on the tube label.
- Use only certified RNase-free tubes and reagents.

RNAGEM gives linear yields for 10 to $\sim 10^5$ 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 10x SILVER buffer.

Extraction Volume	Cell numbers	Volume of RNAGEM™
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.

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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 SILVER buffer before use.

Cells can be collected directly in the extraction reagent mastermix or using ZyGEM reagents within one hour of preparation. For longer periods, reagents should be frozen.

FACS and LCM
Cells can be collected directly in the extraction reagent mastermix or using ZyGEM reagents within one hour of preparation. For longer periods, reagents should be frozen. Alternatively, the pellet can be resuspended in 1X SILVER buffer and an appropriate quantity added to the extraction.

Cell pellets
Up to 5×10^7 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.

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 RNAGEM™ extraction reagents.

Adherent cells
1. Dislodge cells by preferred method (Trypsin or cell scraper).
2. Centrifuge suspension at 200 x g for 5 mins.
3. Remove all of the liquid (a quick spin on a bench centrifuge can help to gather the last few drops).
4. Resuspend the pellet in RNAGEM™ extraction reagents.

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 RNAGEM™ extraction reagents.

Handling different culture types

- The method, the 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@zygem.com.

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 RNAGEM
 - 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 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). 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 NH₄OAc/ethanol (0.1 volumes of 5 M NH₄OAc, and 2.5 volumes 100% ethanol) and stored at -20°C or below.
- Absorbance 260/280 nm is an ineffective quantitation method with RNAGEM-prepared nucleic acids. See website for details.

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