MicroGEM

Antarctica - the source of RNAGEM™ (Supply delivery - Wright Valley, McMurdo Dry Valleys)

RNAGEM - a kit from MicroGEM for extracting RNA from cell culture

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Introduction

RNAGEM has been developed for rapid extraction of RNA. It is ideal for preparing RNA from mammalian cell culture, laser capture micro-dissections and FACS-prepared cell populations.

Most methods for extracting RNA from cell culture or small tissue samples rely on solid phase purification or solvent based extraction and precipitation. In some cases, lysis is achieved using agents that must be neutralised or removed from the solution before the nucleic acid is usable for analysis. This requirement increases the number of manipulations; and if solid phase or solvent methods are used, yields are reduced and bias can be introduced into the population of mRNAs.

The enzymes and reagents used in MicroGEM kits have been selected to be fully compatible with most downstream applications. The result is an RNA extract that is immediately ready for use.

RNAGEM uses a rapid, single-step protocol that releases RNA and DNA with excellent linearity across a wide range of cell numbers. The method is automatable, closed-tube and does not require further purification of the RNA for accurate RT-qPCR analysis. The RNAGEM reagents efficiently lyse the cells and strip protein complexes from nucleic acids, thereby allowing higher processivity of polymerases. The result is greater sensitivity - especially with low abundance transcripts. Reduced handling, and efficient template preparation means that the RNAGEM kits generate mRNA profiles that are as close to the biological reality of the sample as possible.

RNAGEM produces RNA that is suitable for direct use. Figure 1 compares the two RNAGEM methods with that for Trizol®. Both of the RNAGEM procedures are significantly simpler than Trizol and do not use toxic chemicals.

Procedure

1. Add:
   - Cell suspension or pellet
   - 10x Buffer **BLUE**
   - RNAGEM
   - Water
2. Vortex and incubate at 75°C
   < 50,000 cells - 5 min
   > 50,000 cells - 10 min
   A thermal cycler should be used for this step.

DNAse treatment (optional)

1. To the extract add:
   - 10x DNAse buffer
   - DNAse I
2. Incubate:
   37°C for 5 minutes
   75°C for 5 minutes
3. Add 10 x TE Buffer (provided) and store at -20°C or below.

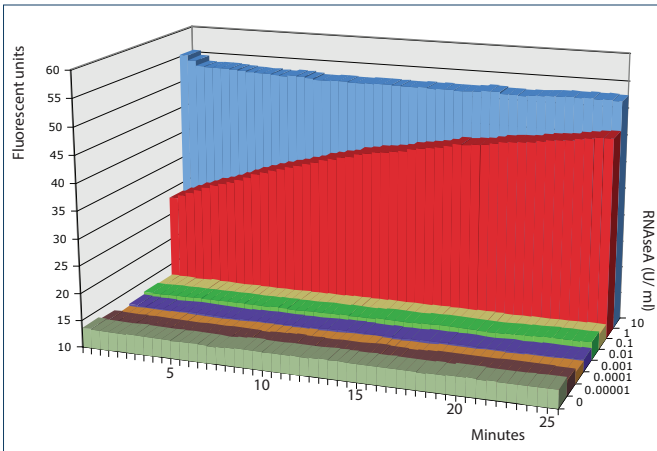
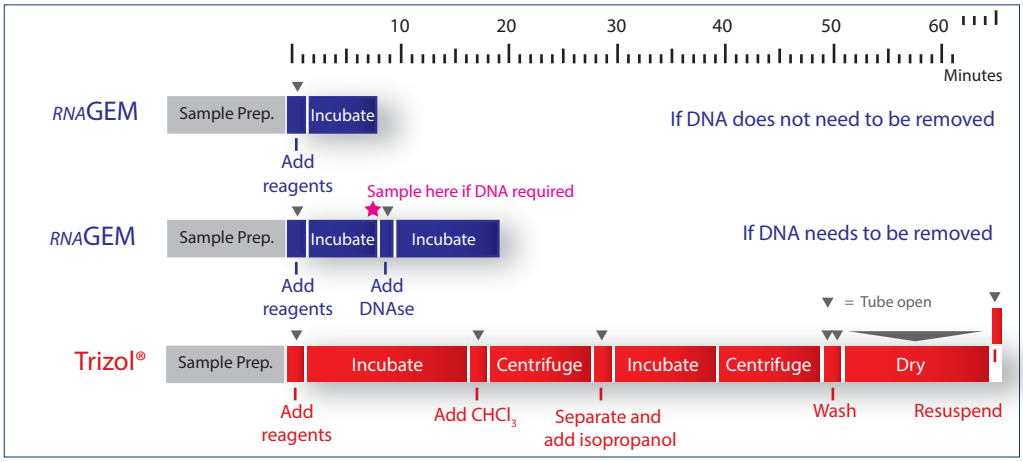


Figure 2. Rates of activity of serial dilutions of RNAse that have been treated with RNAGEM. Y axis = relative fluorescence. X Axis = minutes, Z axis = number of Kunitz Units of RNAse A.

Figure 1. Comparison of the workflows for RNA extraction using RNAGEM and Trizol®



RNAGEM Features

- Simple, closed-tube procedure
- Automation compliant
- No toxic reagents
- Stable at room temperature
- Low cost

* See MicroGEM Application Note 008 for suggestions on how DNA can be used to normalise RNA samples.

Ribonucleases

All MicroGEM reagents are rigorously QC tested for undesirable activities and contaminants.

*RNA*GEM is a powerful, broad-specificity, thermophilic proteinase that aggressively destroys ribonucleases. Figure 2 shows the ability of *RNA*GEM to remove RNAse A at concentrations that far exceed activity levels that would be expected from a biological sample. Typically, no RNAse residual activity can be detected in extracts generated from cell lines and so RNAse Inhibitor should not be needed. However, if the need arises, RNAse inhibitor can be added directly to the extraction cocktail. Human placental RNAse Inhibitor is a robust protein that is resistant to proteolysis and will survive the extraction process.

Scalability and small sample sizes

The simplicity of the method results in high, linear yields over a wide range of sample sizes.

The method provided with the kit is designed for cell numbers of less than 100,000 per extraction, although the procedure can be scaled for larger numbers. Typically an extraction would use the following volumes and incubation times.

Cells	Volume	*RNA*GEM	Incubation time
50,000-100,000	50 - 100 µl	1 µl	10 min
5000-50,000	20 - 50 µl	1 µl	5 min
100-5000	5 - 20 µl	0.5 µl	5 min
<100	1 - 15 µl	0.2 µl	5 min

For cell numbers greater than 100,000, extractions should be scaled upwards from the 50,000-100,000 figures while keeping the incubation time at 10 minutes. The lowest practical reaction volume that can be used for larger cell densities is generally limited by the viscosity of the extract. This is caused by the presence of very high molecular weight DNA and can be reduced by DNAse treatment, vortexing or repeated pipetting to shear the DNA.

The lowest achievable volume for extraction when working with low cell numbers is limited only by evaporation. With specialised equipment sub-µl extractions are possible.

Kit components

- *RNA*GEM enzyme
- 10 x BLUE buffer
- 10 x TE storage buffer
- RNAse-free DNAse 1
- 10 x DNAse Buffer

For more information visit: microgembio.com
or email: info@microgembio.com

*RNA*GEM and RT-PCR

The **BLUE** buffer used by *RNA*GEM has been formulated to be compatible with polymerases, reverse-transcriptases and many other nucleic acid modifying enzymes, and so the extracts can be used without purification in PCR, RT-PCR, qPCR and RT-qPCR.

Figure 3 shows plots obtained when 5 µl of *RNA*GEM extracts were added directly to an RT-qPCR. HeLa cell numbers from 10-50,000 were *RNA*GEM-treated and plots generated from a high abundance mRNA (ACTB; ß-actin) and a low abundance mRNA (BRCA1; breast cancer early onset). The clean traces with gradients similar to the standards demonstrate the lack of inhibition.

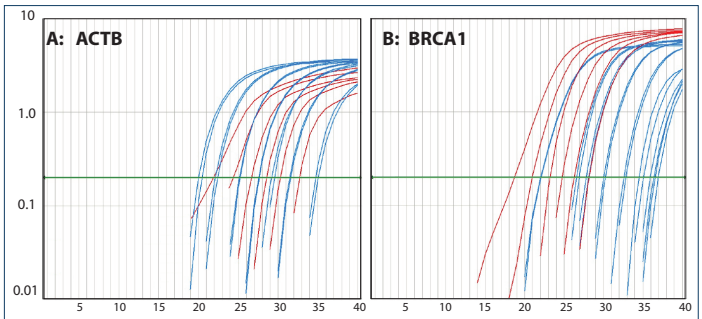


Figure 3. RT-qPCR plots of RNA extracted from a dilution series of HeLa cells from 10 - 50,000 cells. **A:** high copy number mRNA (ACTB). **B:** low copy number mRNA (BRCA1). Red = standards; Blue = duplicate mRNA curves

Using the recommended method, the extraction efficiency of *RNA*GEM is constant over the range of 1 to approximately 100,000 cells (figure 4). Different ranges of linearity can be obtained by small modifications to the base procedure. Advice on modifying the procedure can be obtained from info@microgembio.com.

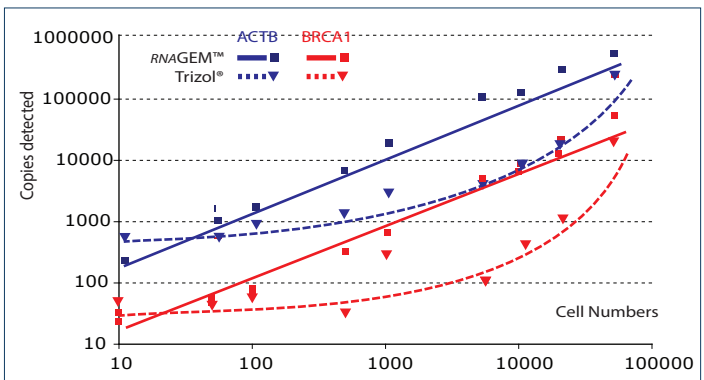


Figure 4. Log/Log plots of HeLa cell numbers versus mRNA copies detected. Both high copy number mRNAs (ACTB) and low copy number (BRCA1) are shown. Two extraction methods are used, *RNA*GEM and Trizol®