MicroGEM

A rapid solution for achieving excellent linearity and high RNA yields from cell culture

Supply Delivery - McMurdo Dry Valleys, Antarctica
Source of ***RNA***GEM™

Introduction

RNAGEM has been developed for rapid extraction of RNA. It is ideal for preparing RNA from mammalian cell culture, laser capture micro-dissection 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.

Rapid, Single-tube Protocol

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

RNAGEM produces RNA suitable for direct use with most downstream applications. Figure 1 compares two ***RNA***GEM methods with Trizol®. Both the ***RNA***GEM procedures are significantly simpler than Trizol and do not use toxic chemicals.

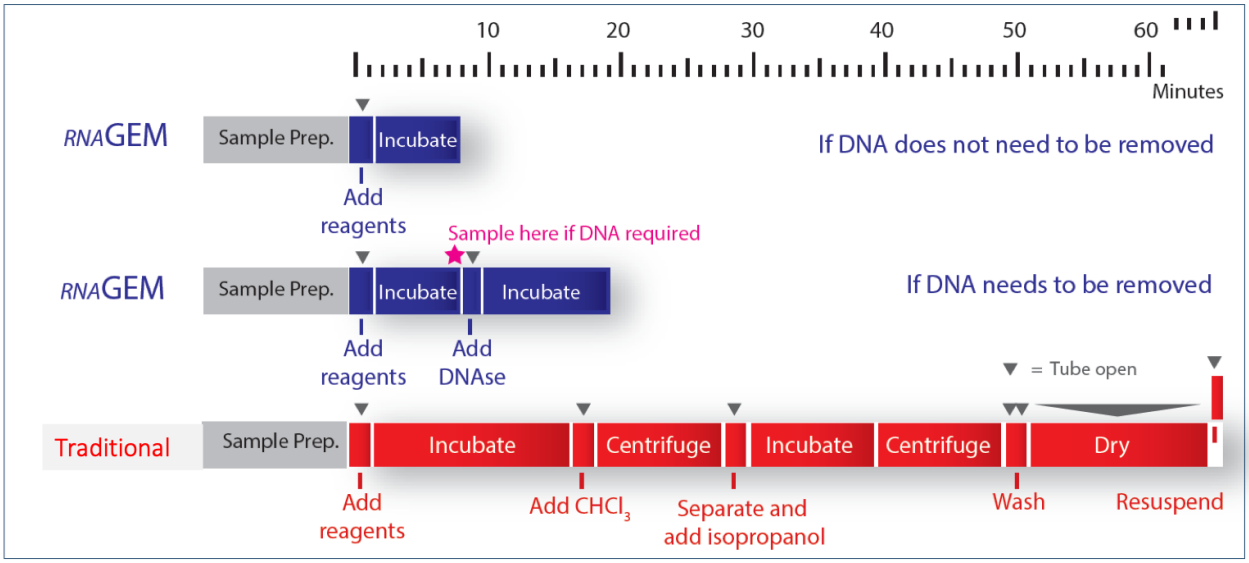

Figure 1. *RNA*GEM workflow vs competitive methods: *The ***RNA***GEM kit allows for total nucleic acid extraction, or just extraction of RNA. The protocol for co-purification of RNA and DNA is rapid, going from sample to nucleic acids in under 20 minutes. Summarized are the co-purification and RNA extraction workflows.*

Kit Components

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

Procedure

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

RNAGEM Benefits

- Simple, closed-tube procedure
- Automation compatible
- No harsh reagents or extra wash steps
- Stable at room temperature
- Rapid, high-quality results

DNAse Treatment (optional)

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

Scalability and Small Sample Sizes

The ***RNA***GEM kit is highly adaptable to a range of extraction volumes and can be scaled from a few cells (where a small volume is desired) to ~ 5 x 10^5 cells. After extraction, an optional DNAse treatment is included to enrich for RNA. The RNA is immediately ready for downstream applications, such as RT-PCR and RT-qPCR. These reagents can be added directly to the RNA sample for a simple, streamlined 96-well plate protocol.

The following volumes and incubation times are examples for a range of extractions:

Cells	Volume	***RNA***GEM	Incubation Time
50,000 – 100,000	50 – 100 µl	1 µl	10 minutes
5,000 – 50,000	20 – 50 µl	1 µl	5 minutes
100 – 5,000	5 – 20 µl	0.5 µl	5 minutes
<100	1 – 15 µl	0.2 µl	5 minutes

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.

RNAGEM and Downstream Applications

The BLUE buffer used by ***RNA***GEM is 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.

The simplicity of the method results in high yields that are linear over a wide range of cell numbers. The plots shown in Figure 2 were obtained when 5 µl of ***RNA***GEM extracts from HeLa cells were added directly to an RT-qPCR reaction. Cell numbers ranging from 10 – 50,000 were extracted with **RNA**GEM 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, and the plots obtained from as few as 10 cells demonstrate the sensitivity of the method with low cell numbers.

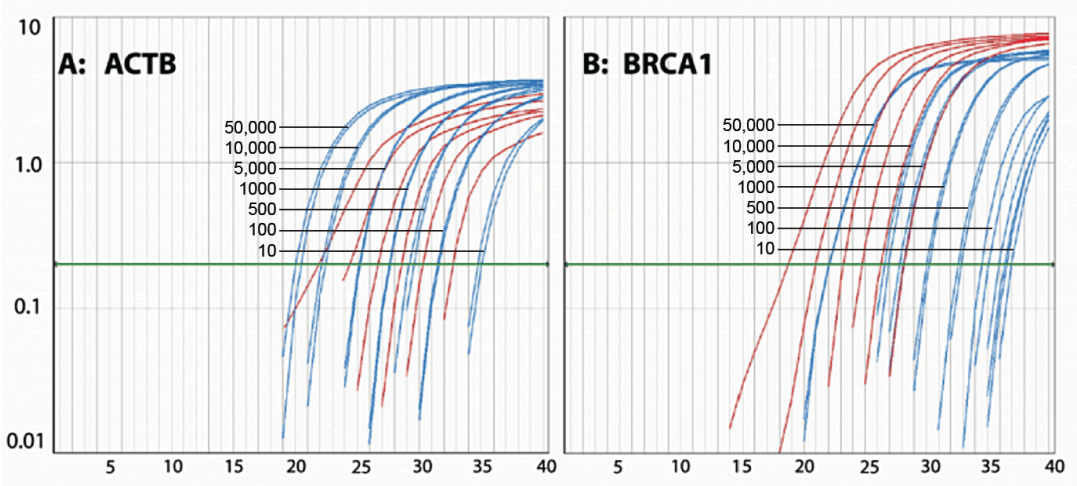

Figure 2. *RT-qPCR plots of RNA extracted from a dilution series of HeLa cells from 10 - 50,000 cells.* **A:** *high copy number mRNA (ACTB)* **B:** *low copy number mRNA (BRCA1). Red = standards; Blue = duplicate mRNA curves.*

The efficiency of ***RNA***GEM extraction is constant over a broad range cell numbers – from single cells to ~ 5 x 10^5. Figure 3 compares ***RNA***GEM's yields to Trizol®. RNAGEM maintains a linear extraction efficiency from low to high cell numbers unlike Trizol which suffers RNA loss with low abundance samples.

RNAGEM releases both DNA and RNA into the extract. Use of intron primers and intron-spanning primers can normalise expression measurements in a single extraction. If it is necessary to remove DNA, use the DNAse provided in the kit.

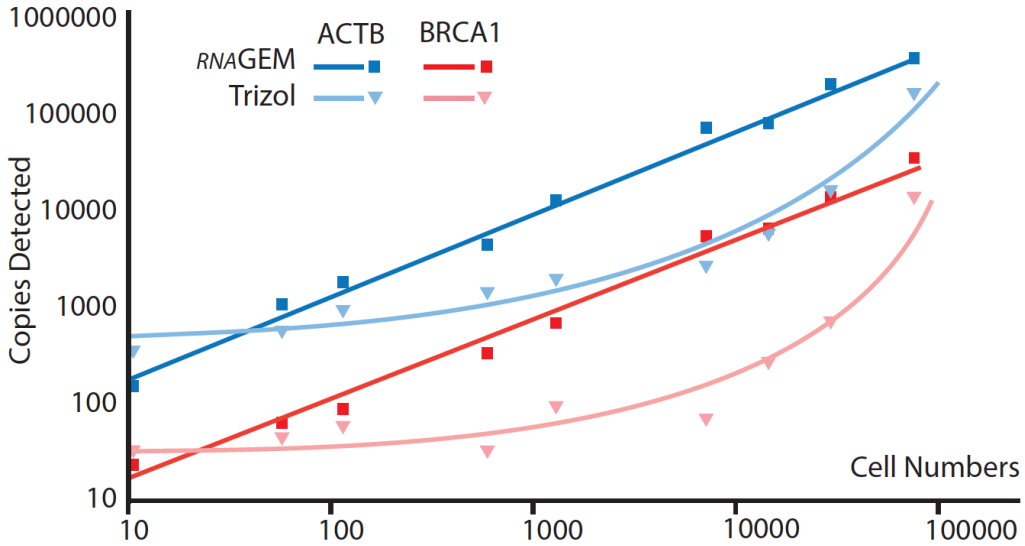

Figure 3. *Log/Log plots of HeLa cell numbers versus mRNA copies detected. Both high copy number mRNAs (ACTB) and low copy number (BRCA1) are shown. Two extraction methods are used: RNAGEM and Trizol.®*

Advantages of Temperature-Driven Enzymatic RNA Extractions

- Simplified, single-tube workflow provides RT-PCR and RT-qPCR ready RNA in 5-10 minutes.
- Releases both RNA and DNA with excellent linearity across a wide range of cell numbers.
- Requires no further purification of the RNA for accurate RT-PCR and RT-qPCR analysis.
- Inhibitor-free means no harsh chemical washes or multiple steps are required.
- Reduced handling means less contamination and no loss of DNA or RNA.
- Perfect for low volume samples.

MicroGEM

At MicroGEM, our goal is to democratize molecular biology, enabling a broader spectrum of users to both employ and benefit from molecular techniques. Our first step toward this goal is the simplification of sample preparation. Our temperature-driven, single-tube process simplifies and reduces the number of steps for traditional nucleic acid extraction, resulting in high-quality extracts with reduced contamination and high yields - all in minutes, not hours.

MicroGEM is committed to minimal packaging and a vibrant, sustainable world.