CELLSCRIPT™
RNA for Translation in Cells

PseudoU-RNA Molecular Weight Markers

Cat. No. **C-PRM101125**

INTRODUCTION

The PseudoU-RNA Molecular Weight Markers are comprised of 7 bands of pseudouridine (Ψ)-containing, single-stranded RNAs of the following sizes: 500, 1,000, 1,500, 2,000, 2,500, 3,000 and 4,000 nucleotides. RNA containing Ψ (Ψ-RNA) in place of the canonical uridine (U-RNA) displays altered mobility during electrophoresis as compared to the comparable U-RNA of identical sequence (see Fig. 1). The PseudoU-RNA Molecular Weight Markers allow users to more accurately estimate the size of Ψ-containing RNAs.

MATERIALS

Materials Supplied

Store at –20°C or –70°C in a freezer without a defrost cycle.

PseudoU-RNA Molecular Weight Markers	
Component	Volume
PseudoU-RNA Molecular Weight Markers in 0.1 mM EDTA.	25 µl

Use 1 µl per lane. 25 lanes per package.
1 µl of the PseudoU-RNA Molecular Weight Markers contains 50 ng/µl of each band.

Inquire about custom kit sizes at 608-442-6484 or sales@cellscript.com.

Materials Required, but not Supplied

- RNA gel loading buffer
- RNase-free, Water or TE Buffer (10 mM Tris-HCl, pH 7.5, 1 mM EDTA)

Fig. 1A

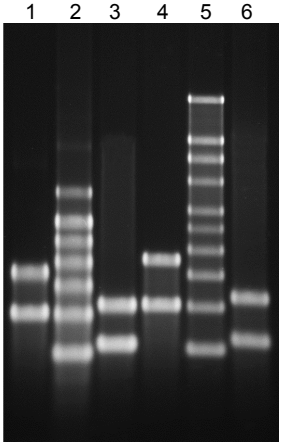

Fig. 1B

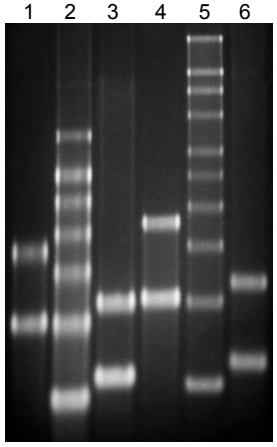

Fig. 1A) 1% denaturing agarose gel.
Fig. 1B) 2% denaturing agarose gel.

The same RNAs were loaded onto each gel.

Lane 1) Ψ-RNAs, 1,875 & 1,077 bases.
Lane 2) PseudoU-RNA Molecular Weight Markers (0.5, 1.0, 1.5, 2,.0, 2.5, 3.0 & 4.0 kilobases).
Lane 3) Ψ-RNAs, 1,203 & 673 bases.
Lane 4) U-RNAs, 1,875 & 1,077 bases.
Lane 5) U-RNA Molecular Weight Markers (0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 5.0, 6.0 & 9.0 kilobases).
Lane 6) U-RNAs, 1,203 & 673 bases.

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SPECIFICATIONS

Storage Buffer

The PseudoU-RNA Molecular Weight Markers are provided in 0.1 mM EDTA.

Functional Testing

The PseudoU-RNA Molecular Weight Markers are functionally tested by denaturing agarose gel electrophoresis analysis compared to a uridine-containing RNA molecular weight markers and discrete transcripts made with either ΨTP or UTP.

Contaminating Activity Assays

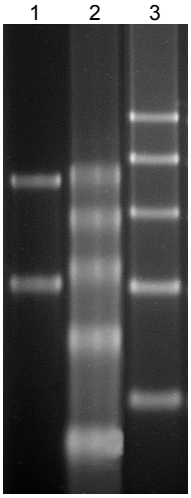
The PseudoU-RNA Molecular Weight Markers are free of detectable RNase activity.

BEFORE YOU START: IMPORTANT TIPS FOR OPTIMAL RESULTS

♦ **Gel Loading Buffers**:

Ψ-RNA displays altered mobility during electrophoresis when run in different gel loading buffers, and sometimes, when run in different batches of the same gel loading buffer (see Fig. 2). Because of this, ***ALWAYS*** make up your PseudoU-RNA Molecular Weight Markers ***AND*** your experimental Ψ-RNA (whose molecular weight is to be determined) with the same tube of the same gel loading buffer.

Figure 2



2% denaturing agarose gel.

Lane 1) Ψ-RNAs, 1,875 & 1,077 bases in "NEW" gel loading buffer.
Lane 2) Partial PseudoU-RNA Molecular Weight Markers (0.5, 1.0, 1.5, 2,.0 & 2.5 kilobases) in "OLD" gel loading buffer.
Lane 3) Partial PseudoU-RNA Molecular Weight Markers (0.5, 1.0, 1.5, 2,.0 & 2.5 kilobases) in "NEW" gel loading buffer.

♦ **Pre Gel Loading RNA Denaturation**:

Pseudouridine stabilizes secondary structure in RNA. Because of this, we strongly recommend a heat denaturation step of **75°C for 10 minutes** in a EDTA-containing gel loading buffer, for the PseudoU-RNA Molecular Weight Markers and the experimental Ψ-RNA prior to loading onto a denaturing agarose gel.

♦ **Maintaining an RNase-Free Environment**:

Highly stable RNases are ubiquitous, including on human skin.

Creating an RNase-free work environment and maintaining RNase-free solutions is critical for successful work with RNA.

We strongly recommend to:

– Use RNase-free tubes and pipette tips.

– Always wear gloves when handling kit components or samples containing RNA and change gloves frequently, especially after touching potential sources of RNase contamination such as door knobs, pens, pencils and human skin. Do not touch any kit component or tube containing RNA with an ungloved hand.

– Keep all kit components tightly sealed when not in use. Keep all tubes containing RNA tightly sealed during the incubation steps.

PROCEDURE

1. Add the reagents in the order listed below. Amounts may be scaled up or down as desired.

Suggested Ψ-RNA MW Markers Procedure	
Component	Amount
RNase-Free Water	x µl
PseudoU-RNA Molecular Weight Markers	1 µl
RNA Gel Loading Buffer to a final conc. of 1X	y µl
Total Reaction Volume	10 µl

! Always make up your PseudoU-Molecular Weight Markers and your experimental Ψ-RNA with the same tube of the same gel loading buffer.

2. Incubate at 75°C for 10 minutes.

3. Load the entire mixture into a well of a denaturing agarose gel.

RELATED PRODUCTS

– INCOGNITO™ SP6 Ψ-RNA Transcription Kit
– INCOGNITO™ T7 5mC- & Ψ-RNA Transcription Kit
– INCOGNITO™ T7 Ψ-RNA Transcription Kit
– INCOGNITO™ T7 ARCA 5mC- & Ψ-RNA Transcription Kit

The performance of this product is guaranteed for one year from the date of purchase.

The Purchaser of this product agrees to the TERMS AND CONDITIONS posted on CELLSCRIPT's website: http://www.cellscript.com.

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