CELLSCRIPT™
RNA for Translation in Cells

EZ-QC™ mRNA Poly(A) Tail Length Assay Kit

Cat. No. **PAT240910**

INTRODUCTION

The **EZ-QC™ mRNA Poly(A) Tail Length Assay Kit** allows one to quantitatively assess the poly(A) tail length of their mRNA production product. 3'-end polyadenylation, the addition of a poly(A) tail to mRNA, is a critical modification that influences mRNA stability and expression levels in eukaryotic cells with longer tails generally providing increased stability and translation efficiency. 3'-poly(A) tails can be added co-transcriptionally (utilizing a template-encoded tail) or post-transcriptionally (via enzymatic addition) to *in vitro* transcribed (IVT) RNA as a step in the mRNA synthesis process. The EZ-QC mRNA Poly(A) Tail Length Assay Kit allows one to forego tedious poly(A) tail length determination methods requiring reverse transcription, PCR or RNase H digests. The kit employs RNase A to digest the synthesized mRNA molecule, leaving the poly(A) tail intact to allow for length determination based upon standard polyacrylamide gel electrophoresis (PAGE) methodology.

For determination of percent capped RNA content, CELLSCRIPT also offers the EZ-QC mRNA Capping Efficiency Assay Kit, EZ-QC XBG mRNA Capping Efficiency Assay Kit and the EZ-QC mRNA Cap 1 Efficiency Assay Kit for complete mRNA characterization.

MATERIALS

Materials Supplied

Important Store at –20°C in a freezer without a defrost cycle. Do not store at –70°C.

EZ-QC™ mRNA Poly(A) Tail Length Assay Kit Contents (10 reactions) Sufficient for 10 experimental and 10 control reactions (optional)	
Kit Component	Reagent Volume
EZ-QC™ RNase A in 50% glycerol, 50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 0.1 mM EDTA and 0.1% Triton® X-100.	20 µl
10X EZ-QC™ RNase A Reaction Buffer 0.6 M Tris-HCl, pH 8.0, 1 M NaCl, 0.1 M EDTA.	20 µl
Stop/Loading Buffer 95% formamide, 10 mM EDTA, pH 7.5, 0.01% Bromophenol Blue and 0.01% Xylene Cyanol.	200 µl
Poly(A) Fluorescence Enhancer, 100X **Protect the tube from exposure to light**.	3 x 1.7 ml
Poly(A) 20-mer Ladder Ready-to-Load: supplied in Stop/Loading Buffer	100 µl
RNase-Free Water	500 µl

Materials Required, but not Supplied (see next page)

⚠ For more information, consult the appropriate safety data sheet (SDS) at www.cellscript.com.

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Materials Required, but not Supplied

- Purified poly(A) tailed mRNA/RNA
- Materials for polyacrylamide gel electrophoresis.
- Materials for polyacrylamide gel visualization, imaging and quantification.

SPECIFICATIONS

Unit Definitions

One standard reaction from the EZ-QC mRNA Poly(A) Tail Length Assay Kit treats 5 pmoles of poly(A) tailed RNA.

Functional Testing

The EZ-QC mRNA Poly(A) Tail Length Assay Kit is functionally tested by treatment of tailed and untailed 1.4-kb RNAs resolved on a 10% polyacrylamide (9.7% acrylamide, 0.3% Bis-acrylamide), 8 M Urea, TBE gel, and quantified using GeneTools software of the captured gel image using a Syngene® G:Box.

Contaminating Activity Assays

All components of the EZ-QC mRNA Poly(A) Tail Length Assay Kit are free of detectable RNase and DNase activities except for the inherent activity of the EZ-QC RNase A.

BEFORE YOU START: IMPORTANT TIPS

♦ **Internal Controls for Optimal Analysis**:

Consideration should be taken to provide adequate internal controls to verify positive results and allow for adequate troubleshooting in the case of atypical findings.

a) The volume of Poly(A) 20-mer Ladder provided allows for the loading of 2 ladders on each gel, one on each side of the controls & transcripts to be tested.

b) Positive Control: mRNA containing a poly(A) tail of a verified length should be included, if available, to verify positive results.

c) Negative Control: RNA **that does not contain a poly(A) tail** should be included, if available, to verify digestion proceeded in a predictable way.

♦ **PAGE Recommendations:**

The standard recommended PAGE system for the EZ-QC mRNA Poly(A) Tail Length Assay is a 10% acrylamide, 8 M Urea, 1X TBE gel, run for 40-50 minutes at 300 volts (constant voltage). This will provide for a size determination range of poly(A) tails of 60-300-A's in length. Shorter and longer electrophoretic run times can be used to resolve poly(A) tail lengths that are shorter than and longer than this range respectively. The provided Poly(A) 20-mer Ladder contains an internal marker band (100 b ssDNA) for proper assignment of the sizes of the Poly(A) 20-mer Ladder bands. Under the standard PAGE conditions, this band will run off of the gel in ~75-90 minutes. Therefore, when running a gel to resolve very long poly(A) tails, load an additional molecular weight marker to allow for proper assignment of the sizes of the Poly(A) 20-mer Ladder bands (e.g., RNA Century™ Markers [Ambion]).

♦ **Maintaining an RNase-Free Environment (prevention of contamination of the stock mRNA sample)**:

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 doorknobs, 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.

♦ **Poly(A) Fluorescence Enhancer, 100X**:

– A precipitate will form in the tube during storage at –20°C. Thawing the tube, warming to room temperature and briefly vortex mixing will bring it back into solution. The Poly(A) Fluorescence Enhancer is stable for a minimum of twenty freeze/thaw cycles.

– Poly(A) Fluorescence Enhancer is somewhat light sensitive. Always store in the dark, but it can be used on the benchtop in the light during gel staining without protection from the light without issues.

PROCEDURE

A. EZ-QC RNase A Treatment Step

1. Combine the following reagents at room temperature in the order indicated below:

EZ-QC mRNA Poly(A) Tail Length Assay Reaction	
Component	Amount
RNase-Free Water	x µl
10X EZ-QC RNase A Reaction Buffer	1 µl
5 pmoles * poly(A) tailed RNA	y µl
EZ-QC RNase A	1 µl
Total Volume	10 µl

* Estimating amount of input RNA/mRNA

pmol/µg ssRNA = 10^6 / (estimated length of the RNA in nucleotides x 330)

for example for a 1,700-nt RNA: 10^6 / (1,700 x 330) = 1.8 pmol/µg

2. Incubate at 37°C for 2 hours, then add 10 µl Stop/Loading Buffer to each reaction.

3. While the reactions are incubating, prepare a 10% acrylamide/8 M Urea/1X TBE gel. Precast 10% Mini-PROTEAN® TBE-Urea Gel: BioRad #4566033, may also be used.
   a) Blow out unpolymerized acrylamide from the wells immediately after comb removal.
   b) Pre-run the gel at 300 volts constant voltage for 20-30 minutes.
   c) Blow out the excess urea from the wells just prior to sample loading.

4. Heat denature the samples at 65–70°C for 5 minutes just prior to gel loading.
   a) Load 2 µl of each stopped reaction mix to the appropriate lanes for PAGE.
   b) For each gel, aliquot 10 µl the Poly(A) 20-mer Ladder into a tube and also heat denature at 65–70°C for 5 minutes just prior to gel loading.
   c) Load 5 µl the Poly(A) 20-mer Ladder in both the first and last lanes to be loaded.

5. Run the gel at 300 volts (constant voltage) for 40-50 minutes.
   a) The Stop/Loading Buffer contains two dyes; bromophenol blue (a darker blue that migrates faster [at ~12-nt in a 10% PAGE]) and xylene cyanol (a lighter blue that migrates more slowly [at ~55-nt in a 10% PAGE]).
   b) The gel should be run until the xylene cyanol dye front is at the bottom of the gel; the bromophenol blue dye will have run off the end of the gel. See PAGE Recommendations (page 3) if running the gel for shorter or longer times.

6. Stain the gel with a dye appropriate for your gel visualization system. Include Poly(A) Fluorescence Enhancer during the staining process.
(e.g., 5 µl SYBR® Gold Nucleic Acid Gel Stain [Invitrogen] and 500 µl Poly(A) Fluorescence Enhancer per 50 ml of deionized water for 30 minutes with slow mixing).

7. Visualize/image the gel. Quantitate relative band intensities (system specific).

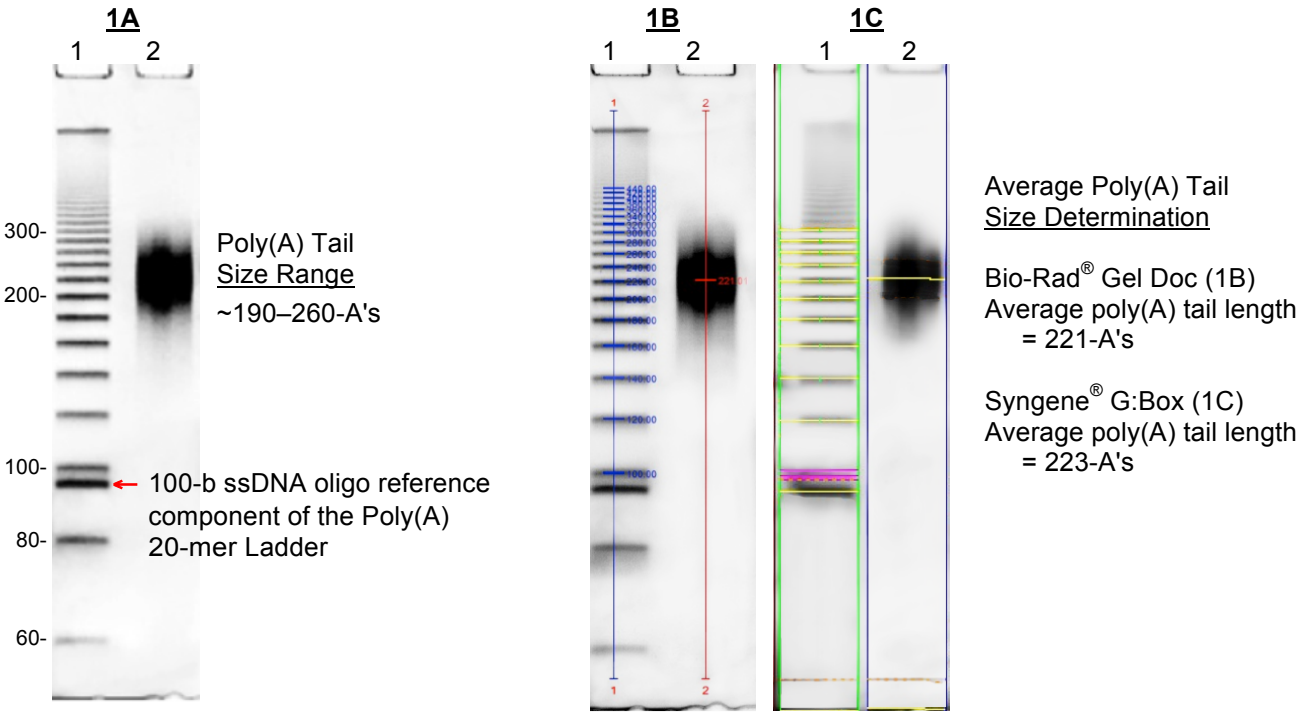
Example Data:

The RNase A-resistant poly(A) tail of treated mRNA will appear as a smear of a defined size range on a polyacrylamide gel (see Figure 1A). This is because Poly(A) Polymerase is a distributive enzyme, meaning that it adds a few A's to the substrate RNA, then dissociates from the substrate and then reassociates to add a few more A's in a cyclic fashion until the reaction is stopped. What is produced is a population of poly(A) tail lengths which when sized, results in a bell curve distribution of tail lengths (see Figures 1A and 2). Gel imaging systems will provide quantitation of the average poly(A) tail length (see Figures 1B and 1C).

Figures 1 A, B, C

10% acrylamide, 8 M Urea, 1X TBE gel stained with SYBR-Gold and Poly(A) Fluorescence Enhancer (5 µl and 500 µl per 50 ml water respectively). Commercially available pre-poured 10% acrylamide gels with urea may also be used (e.g., 10% Mini-PROTEAN® TBE-Urea Gel: BioRad #4566033).

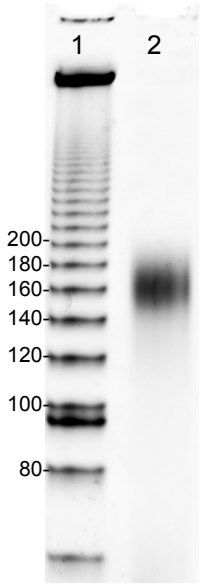
Lanes 1: Poly(A) 20-mer Ladder $(rA_{20})_x$ Lanes 2: RNase A-resistant poly(A) tail of treated mRNA.



Figures 2 A, B

Densitometric data from a PAGE gel [10% acrylamide, 8 M Urea, 1X TBE gel stained with SYBR-Gold and Poly(A) Fluorescence Enhancer (5 μl and 500 μl per 50 ml water respectively)] analyzed using a Syngene G:Box Gel Documentation System.

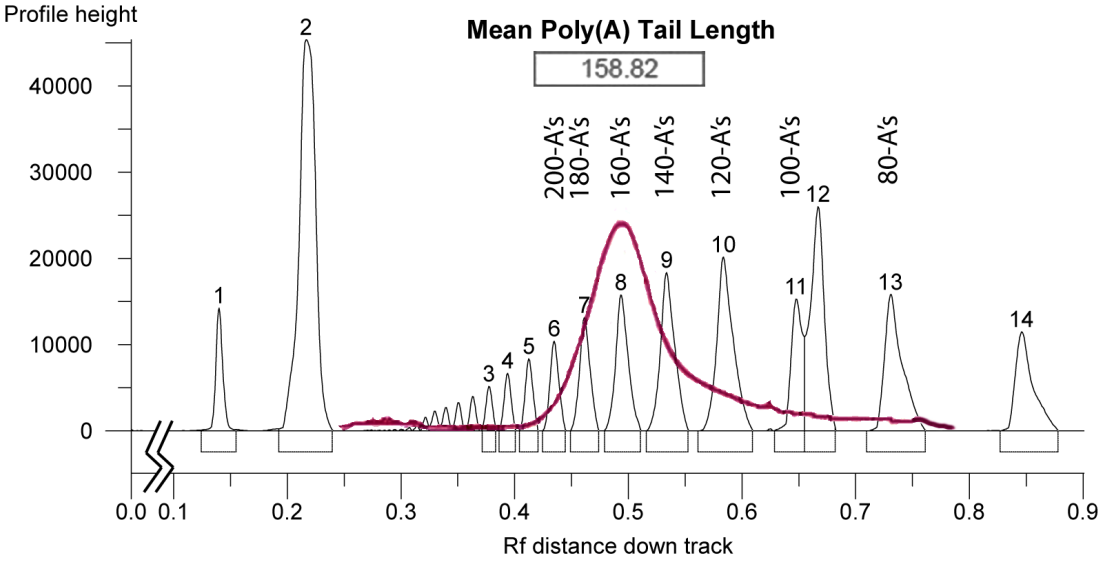
2A



Lane 1: Poly(A) 20-mer Ladder $(rA_{20})_x$

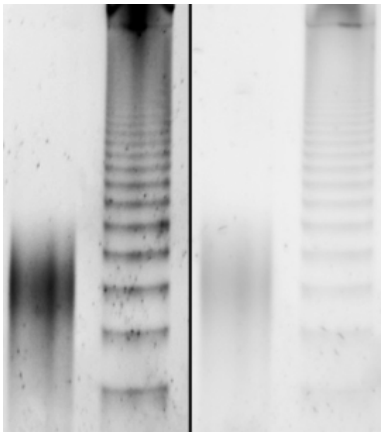
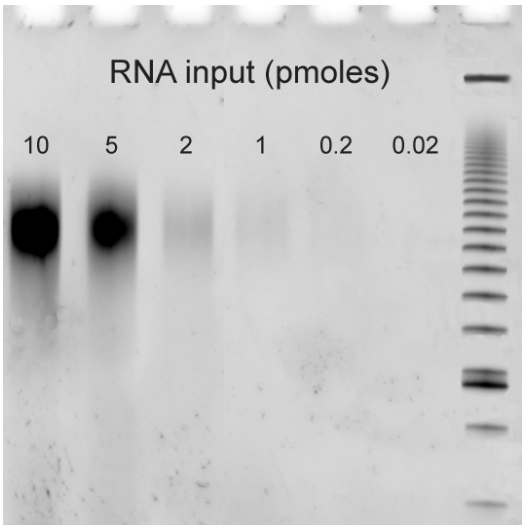
Lane 2: RNase A-resistant poly(A) tails of treated mRNA
Poly(A) Tail Size Range ~190-140-A's

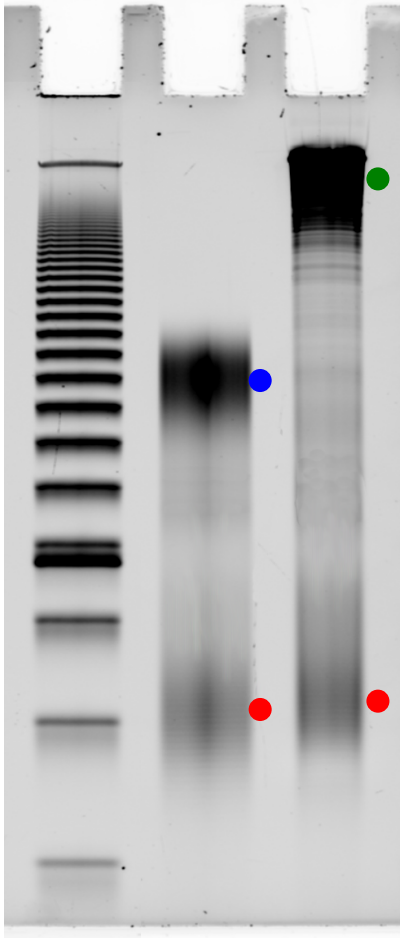
2B



Peaks 6-11) 200, 180, 160, 140, 120, 100-A's respectively
Peak 13) 80-A's
Red Peak) RNase A-resistant poly(A) tails of the mRNA
Size Range ~200-100-A's
Average poly(A) tail length = 159-A's

TROUBLESHOOTING

Symptom	Example	Solution
Light sample **Poly(A) tail band and Poly(A) 20-mer Ladder are both faint**		Poly(A) Fluorescence Enhancer was not used during the gel staining process. • Restain the gel including Poly(A) Fluorescence Enhancer. Insufficient gel staining time. • Return gel to stain solution for a longer period of time. **NOTE**: The 100-b DNA marker band will stain more readily than the poly(A) bands. Use the intensity of this band to determine proper remedy. Excessive dilution of sample with Stop Loading Buffer. • Use a 1:1 ratio of reaction sample and Stop/Loading Buffer. Insufficient sample volume loaded on the gel. • Load ≥2 µl of stopped reaction sample per lane.
Light sample **Light or absent Poly(A) tail bands but Poly(A) 20-mer Ladder is not faint**		Insufficient amount of mRNA used in the reaction. • Use 5 pmol of mRNA in the reaction. Signals generated from ≤ 2 pmol of mRNA produce a faint and harder to identify band. However, Instrumentation may still be sensitive enough to detect at this level. • Load more reaction sample per lane.

Symptom	Example	Solution
Near full lane of many bands		RNase A digestion failure. • Repeat RNase A treatment.
Secondary (smaller) bands seen following digestion		The higher molecular length band is the RNase A-resistant poly(A) tails of the mRNA (blue dot). The lower molecular length band is the RNase A-resistant poly(A) tails of very short secondary (byproduct) transcripts generated during the transcription step of mRNA synthesis (red dots). These small transcripts may be sense (i.e., abortive transcripts) or antisense (generated by RNA-dependent, promoter-independent mechanisms during transcription, and become poly(A) tailed during the tailing step of mRNA synthesis). Running mRNA that has not been treated with RNase A, Lane 3, (green dot) on the gel alongside treated samples (Lane 2) will confirm the presence of tailed short fragments as they are only slightly longer than the RNase A treated version of these fragments. **Note**: Short fragments are not an indication of failure of the EZ-QC mRNA Poly(A) Tail Length Assay, but are instead, a byproduct of *in vitro* transcription.

RELATED PRODUCTS

– A-Plus™ Poly(A) Polymerase Tailing Kit
– EZ-QC™ mRNA Cap 1 Efficiency Assay Kit
– EZ-QC™ mRNA Capping Efficiency Assay Kit
– EZ-QC™ XBG mRNA Capping Efficiency Assay Kit
– INCOGNITO™ T7 mScript™ Complete N1meΨ-mRNA Production System
– INCOGNITO™ T7 mScript™ Complete Ψ-mRNA Production System
– INCOGNITO™ T7 mScript™ Ψ-mRNA Production System
– INCOGNITO™ T7 mScript™ N1meΨ-mRNA Production System
– T7 mScript™ Complete Standard mRNA Production System
– T7 mScript™ Standard mRNA Production System V2

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