CELLSCRIPT™
RNA for Translation in Cells

Assessing mRNA Capping Efficiency and Poly(A) Tail Length with Simple Electrophoretic Separation

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Introduction

Manufacturing high-quality mRNA and performing comprehensive quality control prior to applications is essential for mRNA-based technologies. Accurate measurement of critical quality attributes like 5′ capping efficiency and 3′ poly(A) tail length is required to produce a consistent product. The USP has issued draft guidelines[1] for analysis of these attributes. The test methods suggested include reverse-phase liquid chromatography and mass spectroscopy, requiring special expertise with complex and expensive equipment. We have developed kits that allow for assessment of these mRNA quality attributes using quick and straightforward electrophoretic separation formats, circumventing the expense and limitations associated with more complex technologies and allowing scientists to obtain reliable, same-day results for their mRNA quality control needs.

- **EZ-QC™ mRNA Capping Efficiency Assay Kit (Catalog Number ACE240910)**
- **EZ-QC™ mRNA Cap 1 Efficiency Assay Kit (Catalog Number ONE240910)**
- **EZ-QC™ mRNA Poly(A) Tail Length Assay Kit (Catalog Number PAT240910)**

[1]USP-NF (2024). Analytical Procedures for mRNA Vaccine Quality. Draft guidelines: 3rd Edition.

mRNA Capping

Since only capped RNAs will be expressed in cells, it is important to have the highest possible percentage of capped RNAs present in one's sample. Enzymatic capping is catalyzed by mRNA Capping Enzyme and produces a Cap 0 cap structure. Cap 0 caps can be converted into Cap 1 caps by the additional treatment of an mRNA 2′-O-methyltransferase. Most Cap 1 mRNAs are expressed at higher levels in cells relative to their Cap 0 counterparts since the Cap 1 helps to identify the mRNA as "self" RNA in cells and thus not elicit an innate immune response against the mRNA.

Primary transcript (uncapped) IVT RNA lacks a cap structure.

(5′) pppN$_1$pN(pN)x-OH (3′)

Capped RNAs (Cap 0 and Cap 1) contain an additional N7-methylated guanosine linked by a 5′ to 5′ triphosphate bridge to the 5′-end of the IVT RNA.

m^{7}G(5′)ppp(5′)N1pN(pN)x-OH (3′)

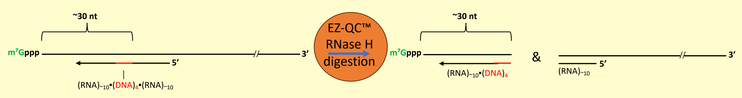
Where m^{7}G represents the 7-methylguanosine cap nucleoside, ppp represents the triphosphate bridge between the 5′ carbons of the cap nucleoside and the first nucleotide of the primary RNA transcript, and N1pN(pN)x-OH (3′) represents the primary RNA transcript, of which N1 is the most 5′ nucleotide. In Cap 1 RNA, this nucleotide contains a 2′-O-methyl group on the ribose moiety.

m^{7}G(5′)ppp(5′)**m$^{2'}$-ON$_1$**pN(pN)x-OH (3′)

EZ-QC™ mRNA Capping Efficiency Assay Kit (Catalog Number ACE240910)

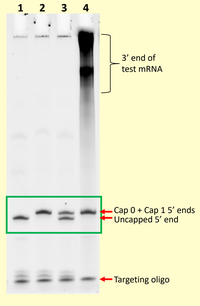
The EZ-QC™ mRNA Capping Efficiency Assay utilizes a chimeric RNA-DNA-RNA Targeting Oligonucleotide (user provided) which hybridizes near the 5′-end of an RNA mixture. This hybridization complex serves as a substrate for EZ-QC™ RNase H cleavage, releasing the capped and uncapped 5′-end fragments which can subsequently be resolved via polyacrylamide gel electrophoresis. Staining with SYBR™ Gold and quantification by gel image band analysis allows for the calculation of the percent capped RNA content of the sample. The EZ-QC™ mRNA Capping Efficiency Assay does not differentiate between Cap 0 and Cap 1 capped RNAs, only between capped (Cap 0 + Cap 1) and uncapped RNAs.

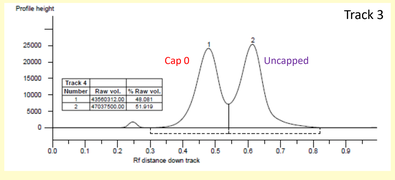
Hybridize the mRNA of interest to a chimeric RNA-DNA-RNA targeting oligo, then digest hybrid with EZ-QC™ RNase H.

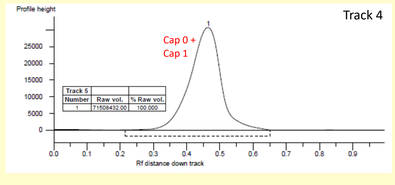


Assess products of digestion via polyacrylamide gel electrophoresis, SYBR™ Gold staining, and image capture.

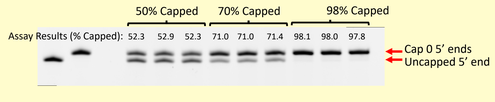
1. Uncapped standard
2. Cap 0 standard
3. 50% Uncapped, 50% Cap 0 standards
4. Fully Capped Test mRNA







EZ-QC™ mRNA Capping Efficiency Assay results are quantitative. Standards were tested in known ratios.

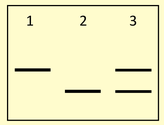


EZ-QC™ mRNA Cap 1 Efficiency Assay Kit (Catalog Number ONE240910)

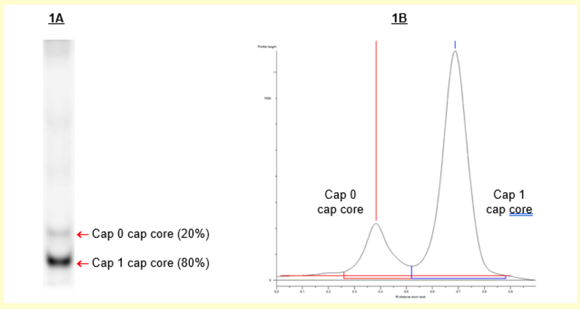
The EZ-QC™ mRNA Cap 1 Efficiency Assay labels mRNA ends with the fluorescent dye Cyanine5 (Cy5). The labeled mRNA is then digested with the EZ-QC™ RNase mixture releasing Cy5-labeled Cap 1 and Cap 0 cap cores. These are resolved using polyacrylamide gel electrophoresis and analyzed using a fluorescent gel imager to measure the relative amounts of Cap 1 to Cap 0 present in the sample. Since mRNA 5′-uncapped ends are not labeled, the EZ-QC™ mRNA Cap 1 Efficiency Assay does not differentiate between uncapped and capped RNAs, but only between Cap 0 and Cap 1 capped RNAs.

	Cap 0 mRNA	Cap 1 mRNA
mRNA	m^{7}G(5′)ppp(5′)N$_1$pN(pN)$_x$-OH (3′)	m^{7}G(5′)ppp(5′)**m$^{2'-O}$**N$_1$pN(pN)$_x$-OH (3′)
⇩ Cy5 Labeling	**Cy5**•m^{7}G(5′)ppp(5′)N$_1$pN(pN)$_x$•**Cy5** (3′)	**Cy5**•m^{7}G(5′)ppp(5′)**m$^{2'-O}$**N$_1$pN(pN)$_x$•**Cy5** (3′)
⇩ RNase Treatment	**Cy5**•m^{7}G(5′)ppp(5′)N$_1$p + Np + … + N•**Cy5**	**Cy5**•m^{7}G(5′)ppp(5′)**m$^{2'-O}$**N$_1$pNp + Np … + N•**Cy5**

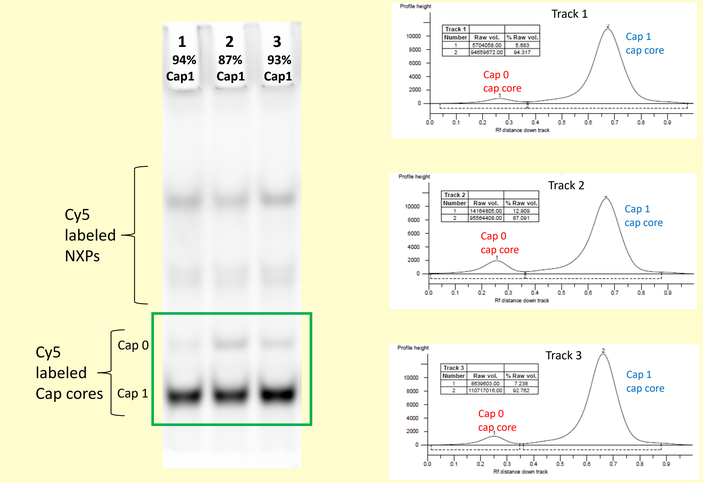
Polyacrylamide gel electrophoresis analysis: Due to a charge difference of the released cap cores, the trinucleotide Cap 1 cap core migrates faster than the dinucleotide Cap 0 cap core.



1. Released Cap 0 cap core: **Cy5**•m^{7}G(5′)ppp(5′)N$_1$p Dinucleotide molecule
2. Released Cap 1 cap core: **Cy5**•m^{7}G(5′)ppp(5′)**m$^{2'-O}$**N$_1$pNp Trinucleotide molecule
3. Mixed sample of Cap 0 & Cap 1 cap cores



Additional example of EZ-QC™ mRNA Cap 1 Efficiency Assay results:

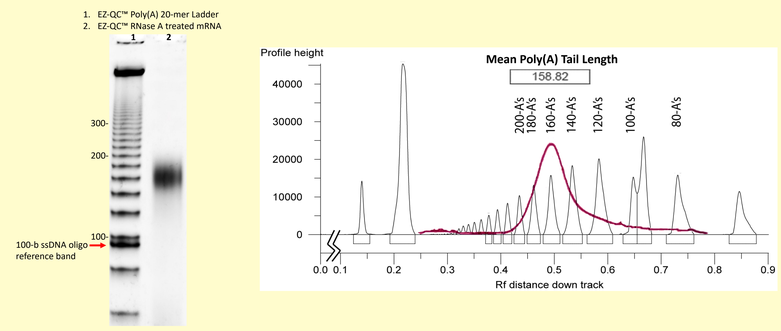


mRNA Poly(A) Tailing

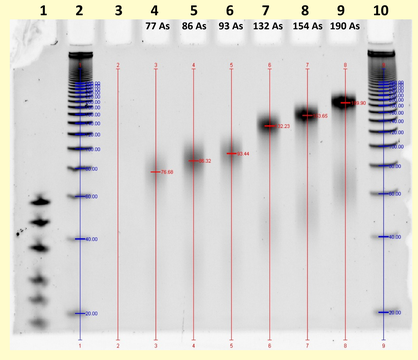
3′-end polyadenylation is a critical modification that influences mRNA stability and expression levels in eukaryotic cells with longer tails generally providing increased stability and translation efficiency. 3′-poly(A) tails can be added co-transcriptionally when encoded by template, or post-transcriptionally via enzymatic addition with poly(A) polymerase.

EZ-QC™ mRNA Poly(A) Tail Length Assay Kit (Catalog Number PAT240910)

The EZ-QC™ mRNA Poly(A) Tail Length Assay employs EZ-QC™ RNase A to digest the mRNA molecule but leave the poly(A) tail intact. In conjunction with the EZ-QC™ Poly(A) 20-mer ladder, the poly(A) tail length can be determined using polyacrylamide gel electrophoresis followed by staining with SYBR™ Gold plus EZ-QC™ Poly(A) fluorescence Enhancer, and gel image analysis.



Additional example of EZ-QC™ mRNA Poly(A) Tail Length Assay results:



1. IDT Oligo 10/60 Ladder (used as reference in this experiment)
2. Poly(A) 20-mer Ladder (100-b ssDNA oligo reference band is absent)
3. Empty Lane
4. 77 A Poly(A) Tail
5. 86 A Poly(A) Tail
6. 93 A Poly(A) Tail
7. 132 A Poly(A) Tail
8. 154 A Poly(A) Tail
9. 190 A Poly(A) Tail
10. Poly(A) 20-mer Ladder (100-b ssDNA oligo reference band is absent)

Conclusion

CELLSCRIPT™'s EZ-QC™ kits facilitate easy, affordable, and accurate methods to determine the 5′ cap and 3′ polyadenylation status of mRNA using standard laboratory equipment for running polyacrylamide gels.

Materials & Methods

All experiments presented here were performed using CELLSCRIPT™ EZ-QC™ Kit protocols and reagents; detailed steps can be found on respective product webpages.

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