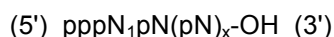


INTRODUCTION

The EZ-QC™ XBG mRNA Capping Efficiency Assay Kit allows one to quantitate the capping efficiency (percent capped RNA content) of Xenopus β-globin (XBG) 5' UTR (untranslated region)-containing mRNA production products. 5'-cap structures can be added co-transcriptionally or post-transcriptionally to *in vitro* transcribed (IVT) RNA to produce Cap 0 and/or Cap 1 structures as a step in the mRNA synthesis process. 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. The EZ-QC XBG mRNA Capping Efficiency Assay Kit allows one to forego tedious and expensive capping efficiency determination methods such as HPLC and mass spectrometry, instead allowing for determination based upon standard polyacrylamide gel electrophoresis (PAGE) methodology.

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



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.



Where m^7G 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 $\text{N}_1\text{pN}(\text{pN})_x\text{-OH} \quad (3')$ represents the primary RNA transcript, of which N_1 is the most 5' nucleotide. In Cap 1 RNA, this nucleotide contains a 2'-O-methyl group on the ribose moiety.

The EZ-QC XBG mRNA Capping Efficiency Assay utilizes a chimeric RNA-DNA-RNA Targeting Oligonucleotide (**kit provided**) which hybridizes to the 5'-end region of an RNA mixture when the mRNA construct contains a Xenopus β-globin 5' UTR. This hybridization complex serves as a substrate for RNase H cleavage releasing the capped and uncapped 5'-end fragments which can subsequently be resolved via PAGE and quantified by gel band analysis allowing for the calculation of the percent capped RNA content of the sample. The EZ-QC XBG 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. Use the EZ-QC mRNA Capping Efficiency Assay Kit (Targeting Oligonucleotide **user provided**) for mRNAs not containing an XBG 5' UTR. For determination of Cap 0 vs Cap 1 content, CELLSCRIPT also offers the EZ-QC mRNA Cap 1 Efficiency Assay Kit as well as the EZ-QC mRNA Poly(A) Tail Length 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™ XBG mRNA Capping Efficiency Assay Kit Contents (10 reactions) Sufficient for 10 experimental (capped samples), 10 experimental control (uncapped samples) and 3 kit control reactions.	
Kit Component	Reagent Volume
EZ-QC™ RNase H in 50% glycerol, 50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 1 mM dithiothreitol (DTT), 0.1 mM EDTA and 0.1% Triton® X-100.	23 µl
10X EZ-QC™ RNase H Reaction Buffer 0.2 M Tris-acetate, pH 7.9, 0.5 M potassium acetate, 0.1 M magnesium acetate and 0.01 M DTT.	23 µl
ScriptGuard™ RNase Inhibitor, 40 U/µl in 50% glycerol, 50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 10 mM DTT, 0.1 mM EDTA and 0.1% Triton X-100.	12 µl
XBG 5' UTR Targeting Oligo, 5 µM (5 pmoles/µl) in RNase-Free Water.	28 µl
XBG 5' UTR Control Mix, 2.5 µM (2.5 pmoles/µl) an 80% capped / 20% uncapped RNA Mix in RNase-Free Water.	6 µl
Stop/Loading Buffer 95% formamide, 10 mM EDTA, pH 7.5, 0.01% Bromophenol Blue and 0.01% Xylene Cyanol.	230 µl
RNase-Free Water	575 µl

Materials Required, but not Supplied

- Purified capped mRNA/RNA
- Materials for polyacrylamide gel electrophoresis.
- Materials for polyacrylamide gel visualization, imaging and quantification.
- Optional: molecular weight marker (e.g., ssDNA 10/60 Ladder [IDT])



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

SPECIFICATIONS

XBG 5' UTR Targeting Oligo

The Xenopus β -globin 5' UTR Targeting Oligo supplied in the kit is an RNA-DNA-RNA chimeric oligonucleotide with the following sequence (r = ribonucleoside, d = deoxyribonucleoside):

5'-rCrUrGrArGrCdTdTdCdTrGrCrArArArArG-3'

XBG 5' UTR Control Mix

The XBG 5' UTR Control Mix contains 80% capped (56 nt) and 20% uncapped (55 nt) RNAs of the following sequence: (G = Cap 0 cap structure):

20% uncapped GGGUAAUACAAGCUUGCUUGUUCUUUUUGCAGAAGCUCAGAAUAAACGCUCAACU-3'
80% capped G•GGGUAAUACAAGCUUGCUUGUUCUUUUUGCAGAAGCUCAGAAUAAACGCUCAACU-3'

Unit Definitions

One standard reaction from the EZ-QC XBG mRNA Capping Efficiency Assay Kit treats 5-10 pmoles of RNA.

One unit of ScriptGuard RNase Inhibitor results in 50% inhibition of 5 ng of RNase A. RNase H is not inhibited by ScriptGuard RNase Inhibitor.

Functional Testing

The EZ-QC XBG mRNA Capping Efficiency Assay Kit is functionally tested by treatment of capped and uncapped 55-nt RNAs resolved on a 20% polyacrylamide (19.5% acrylamide, 0.5% 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 XBG mRNA Capping Efficiency Assay Kit are free of detectable RNase and DNase activities except for the inherent activity of the EZ-QC RNase H.

5'-GGGUAAUACAAGCUUGCUUGUUCUUUUUGCAGAA-3'  released

and

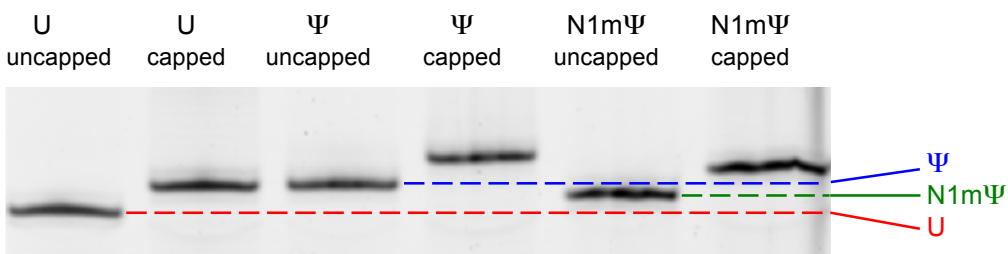
5'-G•GGGUAAUACAAGCUUGCUUGUUCUUUUUGCAGAA-3'  released

EZ-QC mRNA Capping Efficiency Reaction for XBG 5' UTR-containing RNA (continued)

Please note, RNA fragments of identical sequence differing in only whether they contain uridine (U), pseudouridine (Ψ) or N1-methyl-pseudouridine (N1mΨ) do not comigrate on 20% acrylamide/8 M Urea/ 1X TBE gels recommended in this product literature (see Figure 1 below).

The XBG 5' UTR Control Mix included in this kit contains pseudouridine. It is included as an internal control for testing kit performance, not as comigration markers for experimental data that may contain uridine or N1-methyl-pseudouridine or a XBG 5' UTR excised fragments of different lengths.

Figure 1



BEFORE YOU START: IMPORTANT TIPS**◆ RNA Source:**

RNAs can be capped co-transcriptionally using a cap analog molecule during the IVT reaction, or post-transcriptionally (enzymatically) using a Capping Enzyme (e.g., CELLSCRIPT's ScriptCap™ Cap 1 Capping System, T7 mScript™ Standard mRNA Production System or INCOGNITO™ T7 mScript™ Ψ- or N1meΨ-mRNA Production Systems) after mRNA production. **It is important to retain and assay enough of the uncapped IVT RNA product to serve as the uncapped control reaction, along with the experimental capped RNA product.**

◆ Cap 0- vs. Cap 1-mRNA:

The difference between Cap 0- and Cap 1-mRNA is the addition of a methyl group at the 2'-O position of the penultimate (second from the 5' end) nucleotide of the transcript in Cap 1-mRNA. This methylation is part of the natural capping process in higher eukaryotic cells and usually improves *in vivo* translation versus the corresponding Cap 0-mRNA. The EZ-QC XBG 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. For determination of Cap 0 vs Cap 1 content, CELLSCRIPT also offers the EZ-QC mRNA Cap 1 Efficiency Assay Kit.

◆ 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.

It is strongly recommended 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.

PROCEDURE

A. EZ-QC RNase H Treatment Step

- For each reaction, **treat both**, some of the **uncapped** (IVT) RNA that was used to make the capped mRNA, as well as the **capped** mRNA itself. The uncapped RNA sample serves as a necessary control for the analysis.

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

EZ-QC XBG mRNA Capping Efficiency Assay Reaction	
Component	Amount
RNase-Free Water	x μ l
10X EZ-QC RNase H Reaction Buffer	1 μ l
5 μ M XBG 5' UTR Targeting Oligo	1.2 μ l
ScriptGuard RNase Inhibitor	0.5 μ l
5-10 pmoles * capped mRNA, and in a separate control reaction , uncapped IVT RNA	y μ l
EZ-QC RNase H	1 μ l
Total Volume	10 μ l

 Use 5 pmoles (2 μ l) of the XBG 5' UTR Control Mix if running the kit control reaction.

* Estimating amount of input RNA/mRNA

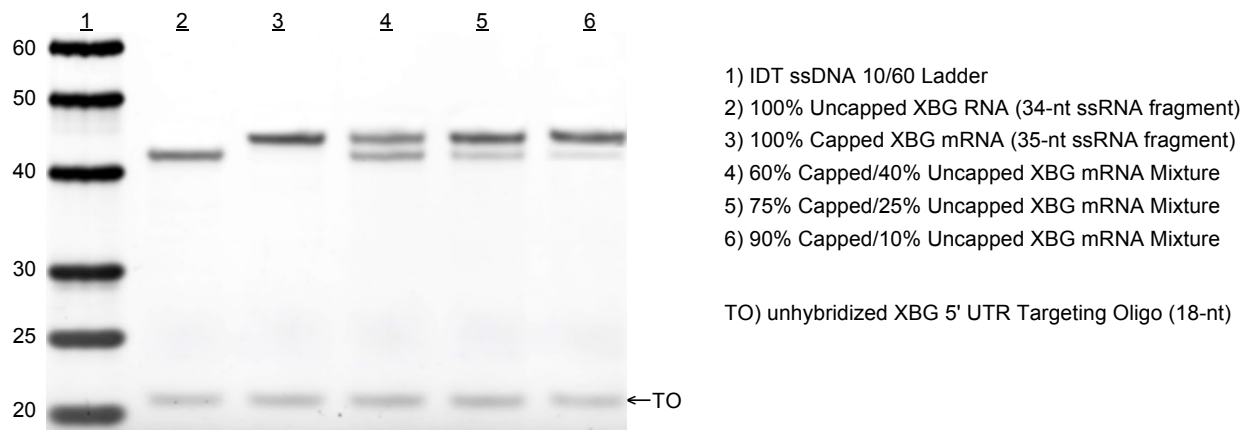
$$\text{pmol}/\mu\text{g ssRNA} = 10^6 / (\text{estimated length of the RNA in nucleotides} \times 330)$$

$$\text{for example, for a 1,700-b RNA: } 10^6 / (1,700 \times 330) = 1.8 \text{ pmol}/\mu\text{g}$$

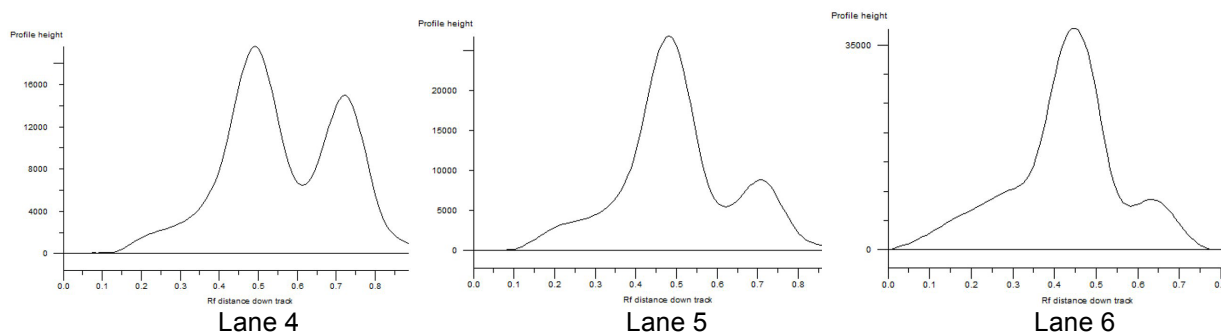
- Incubate at 37°C for 30 minutes, then add 10 μ l Stop/Loading Buffer to each reaction.
- While the reactions are incubating, prepare a 20% acrylamide/8 M Urea/1X TBE gel.
 - Blow out unpolymerized acrylamide from the wells immediately after comb removal.
 - Pre-run the gel at 300 volts constant voltage for 20-30 minutes.
 - Blow out the excess urea from the wells just prior to sample loading.
- Pre-heat denature the samples at 65–70°C for 5 minutes just prior to gel loading.
 - Load 5 μ l of each stopped reaction mix to the appropriate lanes for PAGE.
 - Load the capped sample adjacent to the uncapped control sample.
 - Running a molecular weight marker, while not necessary for analysis, is optional.
- Run the gel at 300 volts (constant voltage) for 2-3 hours.
 - The Stop/Loading Buffer contains two dyes; bromophenol blue (a darker blue that migrates faster) and xylene cyanol (a lighter blue that migrates more slowly).
 - The gel should be run until the xylene cyanol dye front is approximately 1 cm from the end of the gel; the bromophenol blue dye will have run off the end of the gel.
- Stain the gel with a dye appropriate for your gel visualization system (e.g., SYBR® Gold Nucleic Acid Gel Stain [Invitrogen]).
- Visualize/image the gel. Quantitate relative band intensities (system specific).

Example Experimental Data:**Figure 2A**

20% acrylamide, 8 M Urea, 1X TBE gel stained with SYBR-Gold (5 µl per 50 ml water).

**Figure 2B**

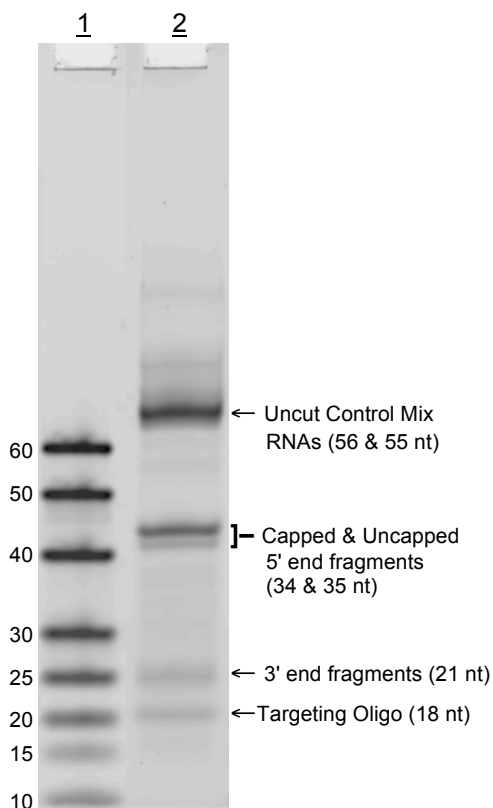
Densitometric data from lanes 4, 5 & 6 from the above PAGE gel using a Syngene G:Box Gel Documentation System.



Lane	Sample	Percent Capped	Percent Uncapped
4	60:40 Mix	58.1	41.9
5	75:25 Mix	77.2	22.8
6	90:10 Mix	91.2	8.8

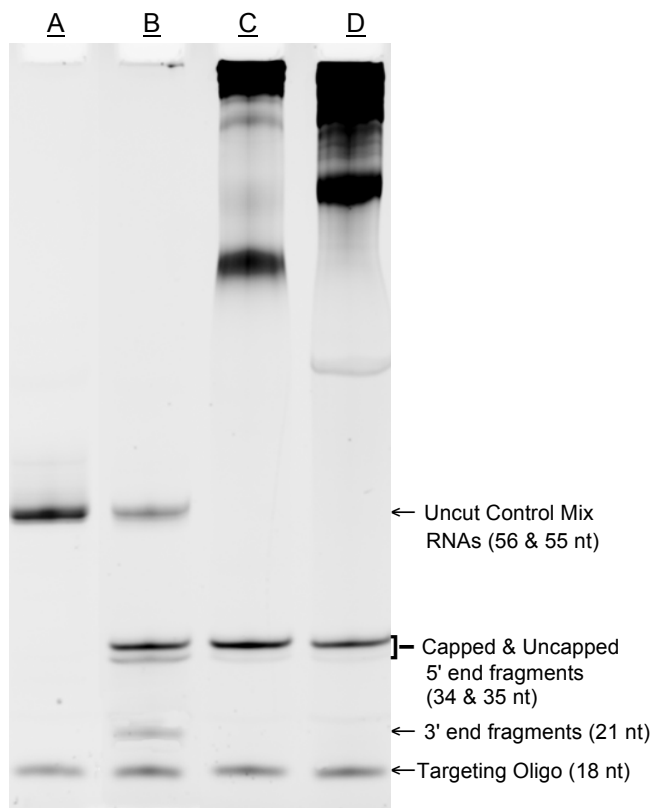
Example XBG 5' UTR Control Mix Reaction Data:Figure 3AFigure 3B

20% acrylamide, 8 M Urea, 1X TBE gel stained with SYBR-Gold (5 µl per 50 ml water).



Lane 1) IDT 10/60 ssDNA Ladder

Lane 2) XBG 5' UTR Control Reaction



Lane A) non-EZ-QC RNase H treated, XBG 5' UTR Control Mix and Targeting Oligo.

Lane B) EZ-QC RNase H treated, XBG 5' UTR Control Mix and Targeting Oligo.

Lane C) EZ-QC RNase H treated, XBG 5' UTR-containing mRNA #1 with Targeting Oligo.

Lane D) EZ-QC RNase H treated, XBG 5' UTR-containing mRNA #2 with Targeting Oligo.

TROUBLESHOOTING

Symptom	Solution
More than two bands present in the capped/uncapped region of the gel	The Targeting Oligo hybridized in multiple locations. Run the capped and uncapped experimental samples alongside a kit control reaction to identify the experimental bands of interest, then quantitate only the bands of interest. Note: the released 5'-end fragments from the experimental mRNAs may not comigrate with those of the control reaction depending on the sequence of the experimental mRNAs.
	Targeting Oligo migrates within the capped/uncapped excised fragment range. Run the capped and uncapped experimental samples alongside a lane containing the XBG 5' UTR Targeting Oligo-only to identify the experimental bands of interest, then quantitate only the bands of interest.
Faint capped/uncapped bands	mRNA 5'-end secondary structure is preventing efficient hybridization of the Targeting Oligo. Preanneal the mRNA and Targeting Oligo, via mixing, heating and cooling, before adding the rest of the reaction components.
	A molar excess of Targeting Oligo was not used in the reaction. Unhybridized Targeting Oligo should be visible on the gel. See image on page 7. - Recheck experimental stoichiometry. - Add more Targeting Oligo to the reaction.
	Load more completed reaction sample per well.
	Increase the reaction incubation time to 60 minutes.
Near full lane of many bands	RNase contamination. - Be sure to use ScriptGuard RNase Inhibitor in the reaction. - Decontaminate tubes, pipet tips and surfaces. Always use gloved hands.
	XBG 5' UTR Targeting Oligo is degrading. Run a sample of the XBG 5' UTR Targeting Oligo-only on a gel to assay for integrity.
Fuzzy bands	Problems with the PAGE system (e.g., gel not totally polymerized, gel ran too hot, urea wasn't blown out of the wells just prior to sample loading, buffer issue). Rerun the gel.
Capped and uncapped bands unresolved	A <20% polyacrylamide gel was used. Rerun the gel using 20% polyacrylamide. Commercially-available pre-poured 15% gels most often do not resolve the bands adequately.

REFERENCES

1. Krieg, P.A. and Melton, D.A. (1984) Nucl. Acids Res. 12, 7057.
2. Patient, R.K. et al., (1983) J. Biol. Chem. 258, 8521.
3. Kruys, V. et al., (1987) Proc. Natl. Acad. Sci. U.S.A. 84, 6030.
4. Genbank Accession M17374.2

RELATED PRODUCTS

- Cap-Clip™ Acid Pyrophosphatase
- EZ-QC™ mRNA Cap 1 Efficiency Assay Kit
- EZ-QC™ mRNA Capping Efficiency Assay Kit
- EZ-QC™ mRNA Poly(A) Tail Length Assay Kit
- INCOGNITO™ T7 mScript™ Complete N1meΨ-mRNA Production System
- INCOGNITO™ T7 mScript™ Complete Ψ-mRNA Production System
- INCOGNITO™ T7 mScript™ N1meΨ-mRNA Production System
- INCOGNITO™ T7 mScript™ Ψ-mRNA Production System
- ScriptCap™ Cap 1 Capping System
- ScriptCap™ m⁷G Capping System
- ScriptCap™ 2'-O-Methyltransferase
- T7 mScript™ Complete Standard mRNA Production System
- T7 mScript™ Standard mRNA Production System V2

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

SYBR is a registered trademark of Molecular Probes, Inc., Eugene, Oregon.

Syngene is a registered trademark of Synoptics Ltd Corp, Cambridge, United Kingdom.

Triton is a registered trademark of Rohm & Haas, Philadelphia, Pennsylvania.

Cap-Clip, CELLSCRIPT, EZ-QC, INCOGNITO, mScript and ScriptCap are trademarks of CELLSCRIPT, Madison, Wisconsin.

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

© 2026 CELLSCRIPT, All rights reserved.