



Get the Highest Yield of 5'-Capped RNA from an *In Vitro* Transcription Reaction

EPICENTRE Biotechnologies' AmpliCap™ T7 & SP6 High Yield Message Maker Kits, AmpliCap-MAX™ T7 & T3 High Yield Message Maker Kits, and MessageMAX™ T7 Capped Message Transcription Kit, are formulated to produce the highest yields of 5'-capped RNA from an *in vitro* transcription (IVT) kit for use in *in vitro* translation, microinjection and mRNA processing studies.

The AmpliCap and AmpliCap-MAX Kits feature an optimized PreMix solution of NTPs and the standard m⁷G[5']pppp[5']G cap analog commonly used in eukaryotic translation studies. The AmpliCap-MAX T7 reaction typically produces higher yields of 5'-capped RNA using a shorter reaction time (30 minutes) than an AmpliCap T7 reaction (Table 1).

Continued on next page

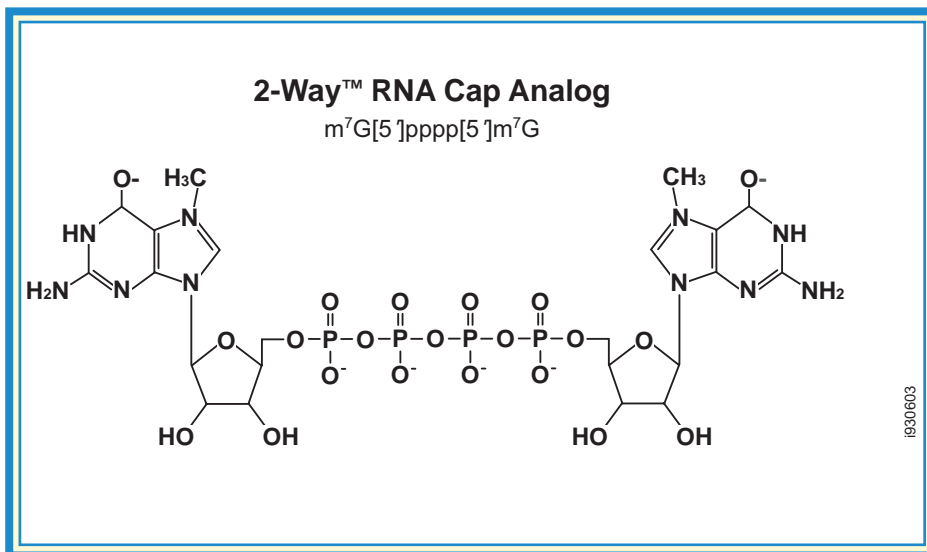


FIG 1. EPICENTRE's new symmetrical dimethylated 2-Way™ RNA Cap Analog m⁷G[5']pppp[5']m⁷G.

Extraction of Rotavirus RNA—Continued from page 19

high quality nucleic acids that can serve as templates in RT-PCR.

In order to determine the 'serotype' of the rotavirus strain by RT-PCR, six serotype-specific forward primers were used in individual RT-PCR molecular typing experiments in combination with a common reverse primer. The results showed an amplicon of size ~750 bp, with serotype 1 specific primers (FIG 1B, lane 3), and none with the other serotype specific primers (FIG 1B, lanes 4-8). A product of similar size was also seen in reactions that contained a mixture of six serotype-specific forward primers and a common reverse primer in the same tube (FIG 1B, lane 9). This demonstrates that molecular typing of rotavirus can be performed using one, single tube RT-PCR reaction.

The MasterAmp™ RT-PCR Kit for High Sensitivity also yielded a single product of ~750 bp in reactions that contained a mixture of six primers and a common reverse primer (FIG 1B, lane 10). As the RetroAmp™ RT DNA Polymerase (EPICENTRE) included in this kit is stable at high temperatures, one-step RT-PCR

typing experiments can be performed on these samples.

Conclusion

EPICENTRE's ExtractMaster™ Fecal DNA Extraction Kit is effective for the isolation of dsRNA from fecal samples, enabling the rapid analysis of potential pathogens in disease outbreaks.

References

1. Widdowson, M. A. *et al.*, (2005) *Curr. Opin. Gastroenterol.* **21**(1), 26.
2. Green, K.Y. *et al.*, (1988) *J. Virol.* **62**(5), 1819.
3. Gouvea, V. *et al.*, (1990) *J. Clin. Microbiol.* **28**(2), 276.

* EPICENTRE's PCR products are sold under licensing arrangements with F. Hoffmann-La Roche Ltd., Roche Molecular Systems, Inc., and Applied Biosystems. The products containing a thermostable DNA polymerase are accompanied by a limited license to use it in the Polymerase Chain Reaction (PCR) and RT-PCR for life science research in conjunction with a thermal cycler whose use in the automated performance of the PCR process is covered by the up-front license fee, either by payment to Applied Biosystems or as purchased, i.e., an authorized thermal cycler.

www.EpiBio.com/extractmaster.asp

ExtractMaster™ Fecal DNA Extraction Kit

FD05005	5 Purifications
FD05025	25 Purifications

Contents: Fecal DNA Extraction Buffer, Proteinase K, Fecal Lysis Buffer, Protein Precipitation Reagent, DNA Precipitation Solution, Pellet Wash Solution, TE Buffer, Inhibitor Removal Resin and Spin Columns.

www.EpiBio.com/monsterscript.asp

MonsterScript™ Reverse Transcriptase

MSTA5110	10 Reactions
MSTA5124	24 Reactions

Includes MonsterScript™ 5X Reaction Buffer.

www.EpiBio.com/high_sensitivity_rt_pcr.asp

MasterAmp™ RT-PCR Kit for High Sensitivity

RT71225	25 Reactions
RT712100	100 Reactions

Contents: RetroAmp™ RT DNA Polymerase, 20X RT-PCR Buffer, MasterAmp™ 10X PCR Enhancer, 25 mM MgCl₂, 25 mM MnSO₄, dNTP Mix - 2.5 mM each, Control Template and Primer Mix, and Sterile Water.

Highest Yield of 5'-Capped RNA—continued from page 20

EPICENTRE's new MessageMAX™ T7 Capped Message Transcription Kit includes the new 2-Way™ Cap Analog (m⁷G[5']pppp[5']m⁷G). This 2-Way Cap Analog is a symmetrical dimethylated molecule (see FIG 1, p. 20), which means that it is always incorporated in the correct orientation during IVT. The 2-Way Cap Analog has also been demonstrated to translate *in vitro*-synthesized luciferase mRNA in a rabbit reticulocyte lysate system >3-fold more efficiently than the same mRNA capped with the standard m⁷G[5']ppp[5']G cap analog.¹

Regardless of the cap analog incorporated, if the capped RNA is used for translation studies, translational efficiencies can be much higher if the mRNA is also 3'-polyadenylated. RNA can be easily and efficiently 3'-polyadenylated using EPICENTRE's A-Plus™ Poly(A) Polymerase Tailing Kit (p. 8).

The Largest Selection of Function-Tested Cap Analogs

EPICENTRE offers the largest selection of cap analogs for *in vitro* transcription of 5'-capped RNAs. Each cap analog is function tested as a substrate for capping an RNA transcript synthesized in an *in vitro* transcription reaction and provided as a 20 mM solution. The new anti-reverse cap analog (ARCA) 3'-O-methyl-m⁷G[5']ppp[5']G, and the new symmetrical 2-Way Cap Analog, m⁷G[5']pppp[5']m⁷G, are unique in that 100% of the capped transcripts produced with these analogs have the cap in the proper orientation for maximum translation efficiency.^{1,2}

References

1. Grudzien, E. *et al.*, (2004) *RNA* 10(9), 1479.
2. Stepinski, J. *et al.*, (2001) *RNA* 7(10), 1486.

Table 1. Comparison of three of EPICENTRE Biotechnologies' *in vitro* transcription kits for producing 5'-capped mRNA. The yield of capped RNA was determined by transcribing 1 µg of the control DNA template provided in each kit.

Kit Features	AmpliCap™ T7 & SP6 High Yield Message Maker Kits	AmpliCap-MAX™ T7 & T3 High Yield Message Maker Kits	MessageMAX™ T7 Capped Message Transcription Kit
<i>In vitro</i> transcription promoter(s)	T7 or SP6	T7 or T3	T7
Cap analog	m ⁷ G[5']ppp[5']G	m ⁷ G[5']ppp[5']G	m ⁷ G[5']pppp[5']m ⁷ G 2-Way™ RNA Cap Analog
Yield of 5'-capped RNA	45 µg from AmpliCap™ T7 reaction 35 µg from AmpliCap™ SP6 reaction	60 µg from both AmpliCap-MAX™ T7 and T3 reactions	60 µg
Reaction time	2 hours	30 minutes	30 minutes
Optimized NTP/ Cap Analog PreMix included?	Yes	Yes	Yes

www.EpiBio.com/rna_caps_analogs.asp

Standard Cap Analog m⁷G[5']ppp[5']G Solution

C31010 500 nmoles
C61025 1,250 nmoles

2-Way™ Cap Analog m⁷G[5']pppp[5']m⁷G Solution

C41210 500 nmoles

ARCA Cap Analog 3'-O-Methyl-m⁷G[5']ppp[5']G Solution

C50110 500 nmoles

Unmethylated Cap Analog G[5']ppp[5']G Solution

C32010 500 nmoles

Trimethyl Cap Analog m₃^{2,2,7}G[5']ppp[5']G Solution

C06005 250 nmoles

www.EpiBio.com/amplicap.asp

AmpliCap™ T7 High Yield Message Maker Kit

AC0707 25 Reactions

AmpliCap™ SP6 High Yield Message Maker Kit

AC0706 25 Reactions

www.EpiBio.com/acmax.asp

AmpliCap-MAX™ T7 High Yield Message Maker Kit

ACM04037 25 Reactions

AmpliCap-MAX™ T3 High Yield Message Maker Kit

ACM04033 25 Reactions

www.EpiBio.com/mmax.asp

MessageMAX™ T7 Capped Message Transcription Kit

MM50110 10 Reactions