RNA Century™-Plus Marker Templates

Store at –20°C.
Do not store in a frost-free freezer.

Catalog # (P/N):	AM7782
Product Description:	A set of 7 templates ready to use for synthesis of RNA size standards by in vitro transcription.
Concentration:	0.5 mg/mL
Volume:	10 µL
Storage Conditions:	Store at –20°C. This product is shipped at ambient temperature. This in no way affects its high-quality performance. Upon receipt, store at –20°C. Avoid multiple freeze-thaw cycles. ***Do not store in a frost-free freezer.*** Aliquots of the product may be stored short-term at 4°C.
Storage Buffer:	10 mM Tris (pH 7.5), 1 mM EDTA.

USER INFORMATION

General Information:

RNA Century™-Plus Marker Templates are a set of 7 linearized plasmids ready for use as templates in an in vitro transcription reaction for synthesis of RNA size standards. When transcribed with T7 RNA polymerase, these templates will produce 7 transcripts with lengths of 1000, 750, 500, 400, 300, 200 and 100 bases (Figure 1). They can be labeled with radioisotopic, biotinylated, or other nonradioisotopically modified nucleotides and are ideal as size standards for ribonuclease protection assays.

Applications:

Radiolabeled Markers
Add 1 µL (0.5 µg) of RNA Century-Plus Marker Templates to a 20 µL in vitro transcription reaction (Ambion® MAXIscript® Kit, P/N AM1312, is ideal for this purpose) that contains a 0.5 mM concentration of each of the four ribonucleotide triphosphates and 10 units of T7 RNA polymerase. To make radiolabeled markers, we recommend that the RNA Century Markers be synthesized at low specific activity. This means adding only a "trace" amount of radiolabeled nucleotide. RNA markers made in this way will not undergo radiolysis as quickly and should be useful for a month.

In Vitro Transcription Reaction Conditions (20 µL)

X µL	RNase-free dH_2O to make a final volume of 20 µL after all other components are added
2 µL	10X Transcription Buffer (with DTT)
1 µL	10 mM ATP
1 µL	10 mM CTP
1 µL	10 mM GTP
1 µL	10 mM UTP
1 µL	0.5 µg RNA Century™-Plus Marker Templates
1–3 µL	[α-^{32}P]UTP or CTP, 800 Ci/mmol (10 mCi/mL in aqueous solution)
2 µL	T7 RNA Polymerase (10 U/µL)

Incubate reaction at 25–37°C for 1 hr. Add 1 µL RNase-free DNase I (2 U/µL) to the reaction to remove the DNA templates, mix thoroughly, and incubate an additional 15 min at 37°C.

Removal of Unincorporated Nucleotides
While it is not always necessary to remove unincorporated nucleotides, it will help to reduce background and radiolysis. Gel purification is generally not used because of the multiple transcripts produced. Also since these transcripts are usually synthesized under non-limiting nucleotide conditions, interfering prematurely terminated transcripts are rarely seen.

Pass the terminated transcription reaction through a G-25 or G-50 chromatography column. Ambion® NucAway™ Spin Columns (P/N AM10070) are RNase-free and ideal for this purpose.

Alternatively, remove unincorporated radiolabeled nucleotides by ethanol precipitation. Add 1/10 volume of 5 M ammonium acetate and 3 volumes of ethanol. It is generally advisable to add 5 µg of yeast RNA, sheared salmon sperm DNA, glycogen, or linear acrylamide as a carrier prior to the precipitation since the actual mass of synthesized transcript can be quite small.

Gel Analysis of Radiolabeled Markers
After DNase treatment and removal of unincorporated nucleotides, add an equal volume of Gel Loading Buffer II (P/N AM8547, 95% formamide/0.025% xylene cyanol/0.025% bromophenol blue/18 mM EDTA/0.025% SDS) to an aliquot of the transcription products (see below for volumes). Heat the reaction for 3 min at 95°C to inactivate the enzymes and eliminate secondary structure in the transcripts. The radiolabeled markers can be stored at –20°C and used for a month, although background levels will increase. It is not necessary to reheat markers prior to use.

Analyze the transcripts by electrophoresis on a 5% polyacrylamide/8 M urea gel. Relative to the dye markers in the gel loading buffer, the 100 nt RNA marker runs between the xylene cyanol and the bromophenol blue bands, and the other four RNA markers all migrate slower than the upper xylene cyanol band.

Using the radiolabeled RNA Markers resuspended in 20 µL (1X Gel Loading Buffer II):

Desired Exposure Time*	Suggested Volume of Radiolabeled Markers
10 min	10–20 µL
1 hr	1–3 µL
3 hr + intensifying screen	2–5 µL of a 1:10 dilution
Overnight	1–3 µL of a 1:10 dilution
Overnight + intensifying screen	1–5 µL of a 1:50 dilution

* For Kodak XRP-1 X-ray film.

Note: 5000 cpm of the labeled markers (about 1000 cpm/band) will require a 3–4 hour exposure time using an intensifying screen. As the markers increase in age, it is necessary to adjust the volume of markers needed to contain 5000 cpm, due to decay of the radiolabel.

Nonisotopically Labeled Markers
These markers can be labeled non-isotopically (either enzymatically or by synthesizing unlabeled markers and subsequent labeling with the Ambion® BrightStar® Psoralen-Biotin Nonisotopic Labeling Kit, P/N AM1480). For unlabeled or nonisotopically labeled markers, use 1 µL of the reaction to check the transcription products by UV shadowing. Load 10 pg per lane to detect the markers using chemiluminescence detection (BrightStar® BioDetect™ Kit; P/N AM1930).

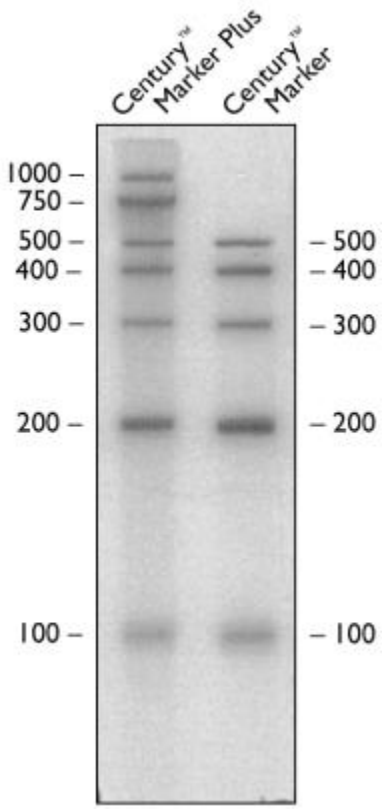

Figure 1. Transcription of RNA Century™ Marker Templates and Century™-Plus Marker Templates. 0.5 µg of each template set was transcribed using the Ambion® MAXIscript® In Vitro Transcription Kit and [α-^{32}P]UTP. 1 µL of each reaction was run on an 8 M Urea, 5% PAGE gel and exposed for 15 min to X-ray film.

QUALITY CONTROL

Functional Testing: 0.5 µg of RNA Century™-Plus Marker Templates is transcribed with T7 RNA Polymerase under standard reaction conditions in the presence of [α-^{32}P]UTP for the production of a low specific activity probe. The set generates 7 distinct bands of the sizes 1000, 750, 500, 400, 300, 200 and 100 bases by autoradiography after separation on a denaturing acrylamide gel. All of the bands will be about equal intensity.

OTHER INFORMATION

Material Safety Data Sheets: Material Safety Data Sheets (MSDSs) can be printed or downloaded from product-specific links on our website at the following address: www.ambion.com/techlib/msds. Alternatively, e-mail your request to MSDS_Inquiry_CCRM@appliedbiosystems.com. Specify the catalog or part number(s) of the product(s), and we will e-mail the associated MSDSs unless you specify a preference for fax delivery. For customers without access to the internet or fax, our technical service department can fulfill MSDS requests placed by telephone or postal mail. (Requests for postal delivery require 1–2 weeks for processing.)

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