

Millennium™ Marker Probe Template

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

Catalog # (P/N):	AM7785
Product Description:	A template for in vitro synthesis of RNA that can be used as a probe for Ambion® RNA Millennium™ Markers (P/N AM7150, AM7151).
Amount:	5 µg
Concentration:	0.5 µg/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-HCl pH 7.5, 1 mM EDTA.

USER INFORMATION

General Information: The Millennium Marker Probe Template is designed as a template for in vitro synthesis of RNA that can be used as a probe for Ambion RNA Millennium Markers in Northern blot hybridizations. The Millennium Marker Probe Template contains a 0.43 kbp insert of bacteriophage lambda DNA (Accession #AMJ02459) within a patented Ambion TRIPLEscript™ vector (US patent #5,547,862), providing sequence complementary to each of the RNA Millennium Markers. Since Ambion TRIPLEscript vectors have tandem SP6, T7, and T3 promoters, any of the corresponding RNA polymerases can be used to synthesize probe for RNA Millennium Markers.

Applications: Use the Millennium Marker Probe Template when a permanent record of the RNA Millennium Markers is desired on the film of a Northern blot. If Millennium Marker probe (MMP) is used in conjunction with a probe for the gene of interest, then it is not necessary to take a picture of the ethidium bromide-stained gel with a fluorescent ruler to determine the mobility of the size markers. After the film of a Northern blot is developed, the length of the mRNA of interest can be estimated.

The MMP can also be used as a control to confirm the transfer of long RNAs to the membrane. Inability to detect the high molecular weight RNA Millennium Markers by Northern blotting is an indication that the transfer to the membrane was poor.

The MMP has been used in Northern blot hybridizations with a variety of total RNA samples from rat, mouse and human. No cross-hybridization of the MMP with the RNA samples was observed.

Transcription Reaction Conditions

To produce the MMP, we recommend using the Ambion MAXIscript® or Strip-EZ® RNA kits (e.g., P/N AM1312, AM1362) to synthesize low specific activity RNA probes. Assemble the reactions at room temperature as indicated below. The reactions are the same as recommended in the Instruction Manuals for MAXIscript and Strip-EZ RNA except that they are formatted to produce low-specific activity RNA probes. For more details, refer to the MAXIscript or Strip-EZ RNA Kit Instruction Manuals, available through the catalog page for these products at www.ambion.com.

<u>Component</u>	<u>MAXIscript Kit</u>	<u>Strip-EZ RNA Kit</u>
Nuclease-free water	10 µL	10 µL
10X Transcription Buffer	2 µL	2 µL
Millennium Marker Probe Template (0.5 mg/mL)	1 µL	1 µL
10 mM ATP	1 µL	1 µL
10mM CTP	1 µL	–
2 mM modified CTP (included in Strip-EZ Kit)	–	1 µL
10 mM GTP	1 µL	1 µL
10 mM UTP	1 µL	1 µL
[α - ^{32}P]UTP or CTP, 800 Ci/mmol	1 µL	–
[α - ^{32}P]UTP, 800 Ci/mmol	–	1 µL
SP6, T7 or T3 RNA Polymerase (10 U/µL)	2 µL	2 µL

Note: Spermidine in the 10X Transcription Buffer can cause precipitation of Millennium Marker Probe Template if the reaction is assembled on ice.

Incubate the reaction at 37°C for 60 min. Add 1 µL of RNase-free DNase (2 U/µL; P/N AM2222) to the reaction and incubate at 37°C for 15 min. The MMP is ready for use.

If desired, remove unincorporated nucleotides by passing the terminated transcription reaction through a G-25 or G-50 chromatograph column. We recommend Ambion® NucAway™ Spin Columns (P/N AM10070) for this purpose.

Hybridization

To allow visualization by ethidium bromide staining and UV fluorescence, load 2 µg of RNA Millennium Markers on the gel along with your RNA samples.

After transferring the RNA to the membrane, use approximately 20,000 TCA-precipitable cpm per mL of hybridization buffer to obtain a good signal in 2–4 hr. Use lower concentrations of Millennium Marker Probe for longer exposures. If you are unable to assay the TCA-precipitable counts, use 1 µL of the transcription reaction per 10 mL of hybridization buffer.

QUALITY CONTROL

Functional Testing:

Linearized Millennium Marker Probe Template plasmid migrates as a single band of the expected size (~3.2 kbp) on a 1% agarose gel. Transcription of the linearized plasmid (0.5 µg) under non-limiting ribonucleotide conditions with 20 units of T7 RNA Polymerase results in at least 15% incorporation of [α -³²P]UTP into product.

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.)

Warranty and Liability:

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