



## Product Information

### RNA MARKER TEMPLATE SET

Product Number **R 4142**

Lot Number 122K1286

Storage Temperature: below  $-20\text{ }^{\circ}\text{C}$

#### Product Description

Concentration: 0.52  $\mu\text{g}/\mu\text{l}$

Storage Buffer: 10 mM Tris, pH 7.5, 1 mM EDTA

Set provides a mixture of 7 DNA templates that upon transcription with T7 RNA polymerase results in 7 transcripts suitable for use as RNA size markers.

The RNA Marker Template set is a mixture of 7 linearized DNA templates, each containing the promoter for T7 RNA polymerase. Transcription with T7 RNA polymerase, results in 7 transcripts of the following lengths: 100, 200, 300, 400, 600, 800, and 1,000 bases. The concentration of each template has been adjusted so all 7 bands are approximately equal in intensity. The transcripts can be labeled with radioisotopes, biotin or any non-radioactive tag compatible with T7 RNA polymerase. These markers are ideal as size standards for single stranded nucleic acid work.

For non-labeled marker, add 0.5  $\mu\text{g}$  of RNA Marker Template Set to a 20  $\mu\text{l}$  reaction containing 0.5 mM of each of the ribonucleotide triphosphates with 10 units of T7 RNA polymerase (Product No. R 0884).

#### Procedure

1. Prepare a 20  $\mu\text{l}$  reaction mix as follows:
  - 2  $\mu\text{l}$  10X Transcription Buffer (400 mM Tris-HCl, pH 8.0, 80 mM  $\text{MgCl}_2$ , 500 mM NaCl, 20 mM spermidine)
  - 1  $\mu\text{l}$  200 mM dithiothreitol
  - 1  $\mu\text{l}$  10 mM ATP
  - 1  $\mu\text{l}$  10 mM GTP
  - 1  $\mu\text{l}$  10 mM UTP
  - 1  $\mu\text{l}$  10 mM CTP
  - 1  $\mu\text{l}$  RNA Marker Template Set (diluted if necessary to 0.5  $\mu\text{g}/\mu\text{l}$ )

1-3  $\mu\text{l}$   $\alpha^{32}\text{P}$ -UTP or -CTP, 800 Ci/mmol (10 mCi/ml in aqueous solution)

1  $\mu\text{l}$  T7 RNA polymerase (10 units/ $\mu\text{l}$ )

Q.S. to 20  $\mu\text{l}$  with RNase-free water (Product No. W 4502)

2. Incubate at  $37\text{ }^{\circ}\text{C}$  for 1 hour.
3. Add 1  $\mu\text{l}$  (2 units/ $\mu\text{l}$ ) RNase-free DNase I (Product No. D 7291) to degrade DNA template, mix well and incubate at  $37\text{ }^{\circ}\text{C}$  for 15 minutes.
4. Add equal volume of gel loading buffer (80% formamide, 0.1% xylene cyanole, 0.1% bromophenol blue, 2 mM EDTA)
5. Heat for 3 minutes at  $95\text{ }^{\circ}\text{C}$  to inactivate the enzyme and denature the transcript.
6. Separate transcripts by electrophoresis on a 5% polyacrylamide/8 M urea gel.

#### Notes

The 100 nucleotide band runs between the xylene cyanole and the bromophenol blue. The remaining bands migrate slower than the xylene cyanole.

The markers can be stored at  $-20\text{ }^{\circ}\text{C}$  for several weeks.

Use of RNase inhibitor may be helpful in transcription reaction.

Reheating markers prior to use is not necessary.

Approximate exposure times for radiolabeled markers:

10 min for 10-20  $\mu\text{l}$

1 hour for 1-3  $\mu\text{l}$

12-16 hours for 1-3  $\mu\text{l}$  of 1:10 dilution

Using an intensifying screen:

3 hours for 2-5  $\mu\text{l}$  of 1:10 dilution

12-16 hours for 1-5  $\mu\text{l}$  of 1:50 dilution

Increase volume of marker loaded on the gel proportionally as the  $^{32}\text{P}$ -label decays.

## References

1. Sambrook, J., et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, p. 10.27- 10.37 (1989).
2. Krieg, P.A. and Melton, D.A. Nucleic Acids Res. **12**, 7057-7070 (1984).
3. Melton, D.A. Proc. Natl. Acad. Sci. USA **82**, 144-148.

1/03