



## Product Information

### RNA MARKER TEMPLATE SET

Product Number **R4142**

Lot Number 040K0668

Storage Temperature: below -20°C

### Product Description

Concentration: 0.81 µg/µl

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 µg of RNA Marker Template Set to a 20 µl reaction containing 0.5 mM of each of the ribonucleotide triphosphates with 10 units of T7 RNA polymerase (Product no. R0884).

### Procedure

1. Prepare a 20 µl reaction mix as follows:
  - 2 µl 10X Transcription Buffer (400 mM Tris-HCl, pH 8.0, 80 mM MgCl<sub>2</sub>, 500 mM NaCl, 20 mM spermidine)
  - 1 µl 200 mM dithiothreitol
  - 1 µl 10 mM ATP
  - 1 µl 10 mM GTP
  - 1 µl 10 mM UTP
  - 1 µl 10 mM CTP
  - 1 µl RNA Marker Template Set (diluted if necessary to 0.5 µg/µl)
  - 1-3 µl α<sup>32</sup>P-UTP or -CTP, 800 Ci/mmol (10 mCi/ml in aqueous solution)
  - 1 µl T7 RNA polymerase (10 units/µl)Q.S. to 20 µl with RNase-free water (Product no. W4502)
2. Incubate at 37°C for 1 hour.

3. Add 1 µl (2 units/µl) RNase-free DNase I (Product no. D7291) to degrade DNA template, mix well and incubate at 37°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°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°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 µl
- 1 hour for 1-3 µl
- 12-16 hours for 1-3 µl of 1:10 dilution

Using an intensifying screen:

- 3 hours for 2-5 µl of 1:10 dilution
- 12-16 hours for 1-5 µl of 1:50 dilution

Increase volume of marker loaded on the gel proportionally as the <sup>32</sup>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.

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