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ProductInformation

RNA MARKER TEMPLATE SET
Product Number **R 4142**
Lot Number 064K1689
Storage Temperature: below –20 °C

Product Description

Concentration: 0.52 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. R 0884).

Procedure

1. Prepare a 20 µl reaction mix as follows:
   2 µl 10X Transcription Buffer (400 mM Tris-HCl, pH 8.0, 80 mM $MgCl_2$, 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^{32}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. W 4502)
2. Incubate at 37 °C for 1 hour.
3. Add 1 µl (2 units/µl) RNase-free DNase I (Product No. D 7291) 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 ^{32}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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