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ProductInformation

RNA Marker template set

Catalog Number **R4142**
Storage Temperature –20 °C

Product Description

The RNA Marker template set provides a mixture of 7 linearized DNA templates. Each template contains the promoter for T7 RNA polymerase and upon 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. The markers are useful for RNase protection assays or as low molecular mass RNA markers for electrophoresis.

The product is supplied as a solution in 10 mM Tris, pH 7.5, with 1 mM EDTA.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

Store the product at –20 °C.

The RNA transcripts can be stored at –20 °C for several weeks and reheating the markers prior to use is not necessary.

Procedure

Non-labeled Markers

For non-labeled markers, add 0.5 µg of the 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 (Catalog Number R0884).

Labeled Markers

1. Prepare a 20 µl reaction mix as follows:
   - 2 µl 10× Transcription Buffer (400 mM Tris-HCl, pH 8.0, with 80 mM $MgCl_2$, 500 mM NaCl, and 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 α-^{32}P-CTP, 800 Ci/mmol (10 mCi/ml in aqueous solution)
   - 1 µl T7 RNA polymerase (10 units/µl)

   Bring final volume to 20 µl with RNase-free water (Catalog Number W4502)
   Note: Use of an RNase inhibitor may be helpful in transcription reaction.

2. Incubate at 37 °C for 1 hour.

3. Add 1 µl (2 units/µl) of RNase-free DNase I (Catalog Number D7291) to degrade the DNA template, mix well, and incubate at 37 °C for 15 minutes.

4. Add an equal volume of gel loading buffer (80% formamide, 0.1% xylene cyanole, 0.1% bromophenol blue, and 2 mM EDTA).

5. Heat for 3 minutes at 95 °C to inactivate the enzyme and denature the transcripts.

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.

Approximate exposure times for radiolabeled markers:

10 minutes for 10-20 µl load
1 hour for 1-3 µl load
12-16 hours for 1-3 µl load of 1:10 dilution

Using an intensifying screen:

3 hours for 2-5 µl load of 1:10 dilution
12-16 hours for 1-5 µl load 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 Press (Cold Spring Harbor, NY: 1989) p. 10.27-10.37.
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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