invitrogen™
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Biotin-14-CTP

Cat. No. 19519-016 **Size: 500 nmol**
Store at -20°C.

Description:
Biotin-14-CTP is a CTP analog that contains biotin attached at the N^4-position via a 14-atom linker. It is provided as a 10 mM solution in 10 mM Tris-HCl (pH 7.5), 0.1 mM EDTA. The amount of material provided is sufficient for up to 20 transcription reactions (1 μg template) using T7 RNA polymerase.

Biotin-labelled RNA can be synthesized by SP6, T3, or T7 RNA polymerase and the appropriate template in the presence of ATP, GTP, UTP, and biotin-14-CTP. Using a 1:1 mixture of biotin-14-CTP:CTP (keeping the total concentration the same as the other nucleotides) will result in a high yield of full length biotin-labelled RNA. Changing the ratio of biotin-14-CTP:CTP to 4:1 (increasing the total concentration of the biotin-14-CTP:CTP mixture to 2.5 times the concentration of each of the other nucleotides) will increase the amount of biotinylation, resulting in the generation of more sensitive probe. Use of the higher biotin-14-CTP:CTP ratio will, however, result in a lower total yield. The biotin-labelled RNA can be detected colorimetrically using Steptavidin-Alkaline Phosphatase Conjugate (Cat. No. 19542-018) and NBT/BCIP (Cat. No. 18280-016) or by chemiluminescence using streptavidin alkaline phosphatase and an appropriate chemiluminescent substrate.

Quality Control:
Purity of biotin-14-CTP is evaluated by reverse phase HPLC. A single peak with >90% of the area must be observed.

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Incorporation of biotin-14-CTP into RNA by T7 RNA polymerase:

The following procedure is for a standard 50-µl reaction using 1 µg of template DNA. For best results, the reaction should be performed under RNAse-free conditions.

1. Combine the following components in a microcentrifuge tube:
   a. Autoclaved distilled (or DEPC-treated) water to bring the total reaction volume to 50 µl.
   b. 5.0 µl of 10X transcription buffer [400 mM Tris-HCl (pH 8.0), 80 mM $MgCl_2$, 250 mM NaCl, 20 mM spermidine]
   c. 2.5 µl of 100 mM dithiothreitol (DTT)
   d. Ribonucleoside triphosphate mixture
      [Final concentration of ATP, GTP, and UTP in the reaction mix is 1 mM and the combined total concentration of the 1:1 biotin-14-CTP: CTP mixture is 1 mM. For incorporating more biotin, a 4:1 mixture of biotin-14-CTP:CTP may be used (combined total concentration of the 4:1 biotin-14-CTP:CTP mixture is 2.5 mM).]
   e. 1 µg of linearized DNA template
   f. >10 units (per 50-µl reaction) of human placental RNase inhibitor
   g. 40-50 units of T7 RNA polymerase
2. Mix thoroughly. Centrifuge briefly to bring the liquid to the bottom of the microcentrifuge tube.
3. Incubate at 37°C for 90 minutes.
4. Stop the reaction by adding 2 µl of DEPC-treated 0.5 M EDTA (pH 8.0).
5. The biotin-labelled RNA may be isolated by ethanol precipitation or gel filtration on Sephadex® G-50.

Biotin-labelled RNA is very stable and under RNAse-free conditions can be stored at -20°C for extended periods. The typical yield of biotinylated RNA using the above condition is greater than 20 µg.

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