Synthesis of Radiolabeled RNA Probes of High Specific Activity

Protocol

This protocol is for the Synthesis of Radiolabeled RNA Probes of High Specific Activity.

1. Linearize template DNA with a restriction enzyme. Extract DNA with phenol/chloroform, then with chloroform/isoamyl alcohol, and precipitate with ethanol. Dissolve DNA in DEPC-treated Water.
2. Combine the following reaction components at room temperature in the order given:

5x Transcription buffer	4 µL
3 NTP Mix, 10 mM each* (without labeled NTP)	1 µL (0.5 mM final concentration)
100 µM CTP	2.4 µL (12 µM final concentration)
[alpha-^{32}P]-CTP, ~ 30 TBq/mmol (800 Ci/mmol)	1.85 MBq (50 µCi)
Linear template DNA	0.2-1.0 µg
Thermo Scientific RiboLock RNase Inhibitor (Cat #EO0381, EO0382, EO034)	0.5 µL (20 U)
T7/T3/SP6 RNA Polymerase (Cat #EP0111, EP0112, EP0113/EP0101, EP0102, EP0103/EP0131, EP0133)	1 µL (20 U)
DEPC-treated Water	to 20 µL
Total volume	**20 µL**

3. Incubate at 37 °C for 2 hours.
4. Stop the reaction by cooling at -20 °C.
5. Determine the percentage of the label incorporated into RNA.

Note:
* To prepare a mix of the three non-labeled NTPs 10 mM each, combine 1 µL of all three NTPs, 100 mM, from the set (Cat #R0481) with 7 µL of DEPC-treated Water. Store the mix at -20 °C for further use.

- Expect specific radioactivity of 3-5 × 108 dpm/µg.
- RNA can be radiolabeled with [^{32}P], [^{35}S] or [3H]-ribonucleotides. Recommended amounts of radiolabeled nucleotides in 20 µL of reaction mixture are as follows: 1.85 MBq (50 µCi) for 5'- [alpha-^{32}P]-CTP, approx. 30 TBq/mmol (800 Ci/mmol); 11.1 MBq (300 µCi) for 5'- [alpha-^{35}S]-UTP, more than 37 TBq/mmol (1000 Ci/mmol); 0.925 MBq (25 µCi) for 5,6- [^{3}H]-UTP, 1.1-2.2 TBq/mmol (30-60 Ci/mmol).
- The yield of the full-length transcripts is reduced when the concentration of labeled NTP is below 12 µM.

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