Radioactive and Non-radioactive DNA Labeling by Nick-translation

Protocol

This protocol is for the Radioactive and Non-radioactive DNA Labeling by Nick-translation.

I. Radioactive DNA labeling by nick-translation

1. Mix the following components:

10x reaction buffer for DNA Polymerase I	2.5 µL
Mixture of 3 dNTPs, 1 mM* (without the labeled dNTP)	1.25 µL
[alpha-32P]-dNTP, ~ 110 TBq/mmol (3000 Ci/mmol)	1.85-3.7 MBq (50-100 µCi)
Thermo Scientific DNase I, RNase-free (Cat #EN0521, EN0523, EN0525) freshly diluted to 0.002 U/µL**	1 µL
Thermo Scientific DNA Polymerase I (Cat #EP0041, EP0042)	0.5-1.5 µL (5-15 U)
Template DNA	0.25 µg
Water, nuclease-free	to 25 µL
Total volume	**to 10 µL**

2. Immediately incubate at 15 °C for 15-60 minutes.
3. Terminate the reaction by adding 1µL of 0.5 M EDTA, pH 8.0.
4. Take an aliquot (1 µL) to determine the efficiency of the label incorporation. A specific activity of DNA at least 10^8 cpm/µg DNA is expected.
5. If needed, the labeled DNA may be separated from the unincorporated radioactive precursors on Sephadex G-50 or Bio-Gel P-60 column or using spin column (Thermo Scientific GeneJET PCR Purification Kit, (Cat #K0701).

Note:
** To prepare a mixture of three non-labeled dNTPs (1 mM of each), mix 1 µL aliquots of stock solutions of each dNTP (100 mM, Thermo Scientific Cat #R0181) with 97 µL of Water, nuclease-free. These dNTP mixes can be stored at -20 °C for further use.** The DNase I, RNase-free can be diluted with the 1x reaction buffer for DNA Polymerase I.*

- The reaction volumes can be scaled up or down providing that the final concentrations of the components (DNA, dNTPs, labeled dNTP) are as indicated in the protocol.
- Radioactive DNA probes with higher specific activities can be prepared using two radioactively labeled dNTPs simultaneously. In this case, the composition of the unlabeled dNTP mix should be adjusted accordingly.

II. Non-radioactive DNA labeling by nick-translation

The protocol above can be used for non-radioactive labeling by nick-translation using biotin-11-dUTP, fluorescein-12-dUTP, DIG-dUTP or aminoallyl-dUTP:

- Normal dTTP is substituted for labeled-dUTP at a molar ratio of 1:3-1:5,
- Reaction time is prolonged to 1-2 hours.

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