

Recommended Protocol for ^3H labeling of DNA

Overview

1. Obtain tritiated SAM: NEB recommends: Perkin Elmer Adenosyl-L methionine, S-[methyl- ^3H]

Code: NET155V001MC (or UC)

15 Ci/mmol (diluted from 78 Ci/mmol), which is about 66 μM .

2. Reaction set-up (add in the following order):

Nuclease-free Water	up to 12 μl
Supplied Methyltransferase Reaction Buffer (10X)	2 μl
Diluted SAM	4 μl
DNA	1 μg
Methyltransferase	4 – 25 units (1 μl)

3. Mix, pipette up and down at least six times
4. Incubate at 37°C for one hour
5. Stop the reaction by heating at 65°C for 20 minutes

Notes

1. The volume of DNA should be 25% or less of the total reaction volume. When using more dilute DNA, increase the reaction volume to 50 μl . Using too much DNA volume in the reaction can cause inhibition by changing the pH or salt concentration of the reaction.
2. The volume of SAM can be increased to 8 μl without inhibition. If more label is required, larger volumes of SAM can be dried in a spin vacuum. The reaction is then set up using the tube containing dried SAM.
3. The incubation time can be increased to 4 hours. Overnight incubations do not give significant increases in methylation.
4. Using a similar protocol, we were able to label 1 μg of Lambda DNA to 3.43×10^6 cpm measured by a standard filter-binding assay. Quenching in the assay was about 40%. (D. Robinson, unpublished observation)