In vitro methylation using Dnmt2 – Tritium-Assay

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reference: Motorin Y, Grosjean H: RNA. 1999 Aug;5(8):1105-18.

reaction buffer (final concentration): 100 mM Tris pH 8.0; 100 mM NH_4OAc (ammonium acetate); 10 mM DTT (freshly added), 0,1 mM EDTA; 10 mM $MgCl_2$;
make a 5x buffer and add fresh DTT from a 100 mM stock just before starting the assay

other solutions needed:
- 5% TCA in H_2O
- 100 % ethanol
- scintillation liquid (ultima gold)

amount of tRNA used per tube: at least 1 µg, better is 3 – 5 µg

reaction volume: depends; usually between 30 and 50 µl

SAM: 7 µM cold SAM (end-concentration) + 1,25 – 1,5 µCi/ sample ^{3}H-SAM
(endconcentration e.g. for 50 µl: 0,025 µCi/µl)
Prepare as a 10x solution in H_2O shortly before starting

Flag-Dnmt2: use about 100 – 200 ng/sample (purified from S2 cells, transfected with Dnmt2-FLAG)

1.) write a pipetting scheme: volumes of RNA, 5x buffer, DTT, SAM, Enzyme and H_2O; for example:

Sample 1

RNA [1 µg/µl]	3 µl
5x buffer	10 µl
DTT [100 mM]	5 µl
10x SAM	5 µl
Enzyme	10 µl
H_2O	17 µl

endvolume: 50 µl

RNA end concentration (1 µg tRNA corresponds to 40 pmol): 2,4 µM

2.) pipet H_2O and RNA in a tube
3.) heat for 90 seconds at 65 °C
4.) add immediately 5x buffer and DTT
5.) at the ^3H work-place, add 10x SAM solution (freshly prepared)
6.) incubate for 2 min at 37 °C
7.) add enzyme, mix well by pipetting
8.) incubate at 37 °C until you want to take the first time point
9.) in the mean time, prepare:
    - 5 % TCA (in H_2O) in a glass on ice
    - prepare small whatman filters, put a pin through one of the corners, stick into styropor-lid
10. take the first time point: pipet 10 – 15 µl on one small whatman filter. Wait for 15 seconds
11. put the filter with the pin into ice-cold 5% TCA, swirl gently
12. continue like this until the last time point has been taken
13. incubate the filters in the 5% TCA solution for additional 10 min
14. wash two times for 10 min in fresh 5% TCA on ice
15. wash once with 100 % ethanol on ice for 5 min
16. pour off the ethanol, dry the filters by sticking the pins back onto the styropor lid for at least 5 min
17. prepare two additional filter papers
18. spot 1 µl of the 10x SAM stock solution on each of the two filters

19. prepare scintillation vials (3 ml of scintillation liquid “ultima gold” per vial)
20. use forceps to put the dried filters into the scintillation vials
21. count the vials in the scintillation counter