

DNA Methyltransferase Assay

Solutions:

Lysis/Assay Buffer - store at 5°Cfinal conc.

for 100 ml:	5.0ml	1M Tris-HCl pH8.0	50 mM
	0.2ml	0.5 M EDTA	1 mM
	10 ml	glycerol	10 %
	1.0ml	Tween 80 (Sigma P-1754)	1 %
	0.1 ml	10% sodium azide	0.01 %

Add fresh just prior to use: DTT to a final concentration of 1mM (1M DTT stock stored at -20°)
PMSF to a final concentration of 1 mM (100mM PMSF in anhydrous isopropanol)

Stop Solution - store at 5°Cfinal conc.

for 100 ml:	0.4 ml	0.5M EDTA	2 mM
	2.5 ml	5 M NaCl	125 mM
	5.0 ml	butanol	5%
	10 ml	10% SDS	10%
	4.0 g	p-aminosalicylate, Na salt (Sigma A-3505)	228 mM
	8.3 ml	3 mg/ml salmon testis DNA, sonicated	250 ug/ml

Add fresh just prior to use: Proteinase K to a final concentration of 1mg/ml
(10 mg/ml H₂O stock stored at -20°)

Other:

Phenol/Chloroform/Isoamyl alcohol (PCI) (25:24:1) - or equivalent

Chloroform/isoamyl alcohol (CI) (24:1) - or equivalent

10% TCA + 3mg/ml BSA

10% TCA

95% ethanol

70% ethanol

2.5 cm² GF-C glass fiber filters

poly (dI-dC)•(dI-dC) - Pharmacia, dilute to 0.25 ug/ul in TE buffer

S-adenosyl-[³H-methyl]methionine (Amersham, TRK 865 - ~85Ci/mmol)*****store at -20°C in aliquots, use within 6- 8 weeks of the batch analysis date****

DNA Methyltransferase Assay**Sample Prep:**

Tissue culture: Cells should be harvested in log-phase growth. Harvest cells, wash twice with ice-cold PBS and count. Transfer cells to eppendorf on second wash. Resuspend cells at $\sim 10^5$ cells/15ul cold lysis buffer and vortex. Do three successive freeze/thaw cycles using a dry ice/ethanol bath and 37°C waterbath. Vortex between cycles. Store at -70°C until ready to do the assay.

Tissue: Homogenize tissue well in cold lysis buffer using tissue homogenizer or by passage through needle. 300 ul should be adequate for small biopsies. Scale volume up for larger chunks. Do three successive freeze/thaw cycles using a dry ice/ethanol bath and 37°C waterbath. Vortex between cycles. Store at -70°C until ready to do the assay.

Assay:

1. Quantify the protein concentration in the sample by the Bradford or other method. Dilute samples in lysis buffer to 0.5- 10ug/15 ul. The assay is linear up to about 4ug total protein/assay for tissue culture cell or 10 ug/assay from tissue specimens. We typically use 1-3 ug/15 ul for cultured cells. Do not overload the assay, the build-up of S-adenosyl homocysteine during the reaction is a feed-back inhibitor of DNA methyltransferases.
2. Add 2 ul poly dI-dC and 3 ul SAM to 15 ul sample for a total volume of 20 ul on ice.
3. Incubate at 37°C for 2 hours.
4. Add 350 ul STOP solution. Incubate 37°C for 30 min.
5. Phenol extract sample (keep aqueous layer!) using an equal volume of P:C:I. Repeat.
6. Chloroform extract sample using an equal volume of C:I
7. Add 700 ul 100% ethanol. Precipitate DNA at -20°C for >1hr. The assay can be stopped here indefinitely.
8. Pellet DNA 17,000g , 20 min.
9. Wash pellet once with 70% ethanol, dry in speed vac or air dry.
10. Resuspend pellet in 30 ul 0.3M NaOH. Incubate 37°C 30 min.
11. Spot onto GF-C filter. Dry filter by baking at 70-80°C in oven or with a heat lamp.
12. Wash filter on vacuum manifold. With vacuum off, Add enough 10% TCA + BSA to cover filter. Wait 10-20 sec. Aspirate. With vacuum on, wash filter with 10% TCA (~5ml), then 95% ethanol

(~5ml). We use a squirt bottle to do this. Other methods for washing the filters should also work.

13. Dry filters as in step 11. (if using scintillant for non-aqueous samples, see below)
14. Transfer to scintillation vial, add 5 ml scintillation fluid, shake and count ^3H channel. Note - you can use scintillation fluid for aqueous or non-aqueous samples using the above method. If you are using scintillant for non-aqueous samples, you must dry the filters at step 13. If you are using scintillant for aqueous samples, it can be omitted. However, you should still dry the filters at step 11 regardless.