

PREPARATION OF TISSUE CULTURE CELLS - H_3 THYMIDINE-PULSED PRIOR TO COUNTING in
Scintillation Counter

Equipment and solutions needed:

- (1) Borrow millipore manifold from Gan's lab.
- (2) Millipore filters 25-30 mm 0.45 μ
- (3) Vacuum with trap
- (4) 0.01 M cold (not tritiated) thymidine in PBS
- (5) 95% EtOH
- (6) 5% TCA (Trichloroacetic acid $CCl_3 COOH$)

Refrigerate to 4°C

- (7) Scintillation vials
- (8) Scintillation fluid (for dry samples)

DO NOT MOUTH PIPET

5 g P.P.O. 2,5-diphenyloxazole Packard #6002025
(scintillation grade M.W. = 221.25)

0.1 g POPOP 1,4-bis-2 (5 Phenyloxazolyl-Benzene) Packard #6002034
(scintillation grade M.W. = 324.37)

in 1 liter Toluene

Scintillation fluid (for aqueous samples)

1 liter Dioxane
1 liter Xylene
0.6 liter Ethanol
208 g Naphthalene
13 g PPO
0.13 g alpha-N-PO

Method- Place filters on manifold; cover unused places with parafilm, wet filters with 1 X PBS, no azide.

Using a 9" Pasteur pipette put contents of tissue culture well onto filter.

Wash 2 times with 0.01M cold (not tritiated) thymidine in PBS.

Vacuum on; wash with cold TCA ~ 5 ml.

Wash with cold 95% ETOH.

* Remove top from vial and drop filter into 5 ml scintillation fluid.

Count

* filters can be dried over low heat & counted as dry samples