

Hypoxia Resistant DNA Synthesis

1. (optional) label cells for 48 hrs with ^{14}C -methyl-Thymidine at 10nCi/ml for 48 hrs
2. Split cells 12 hr prior to expt- 100,000/ 6 cm dish
3. Change media prior to expt- 3 ml/dish
4. Make up thymidine 1 ml/plate with 10 uCi ^3H -methyl Thymidine
5. Place triplicates in anoxia, along with half media
6. Pulse plates for 30 mins with 1 ml thymidine at 4 hr, 8hr, 12hr, 16h, 24 hr with 1 ml thymidine

Collection, Analysis

1. Wash plates X2 with PBS containing 2.5 mM Thymidine
2. Scrape Cells
3. Rinse Whatman glass microfilter filters with PBS. Place filters on manifold
4. Add Cells to filters, slowly turn on vacuum
5. Add 2 ml 10% TCA to filters
6. Rinse with 3 ml cold 100% ethanol.
7. Keep filters on vacuum until dry.
8. Place on Scintillation counter

Counting

1. Use a program with a low energy range (0-18.6) for ^3H and a high energy range (18.6-156) for ^{14}C . Count only ^{14}C labeled cells for correction
2. Determine fraction of ^{14}C that is counted in high energy (around 70%). = ^{14}C ratio.
3. $\text{H-3 counts} = (\text{low energy}) - (\text{high energy}) * (1 - \text{C14 ratio}) / (\text{C14 ratio})$
4. $\text{C14 counts} = (\text{high energy}) / (\text{C-14 ratio})$