

**CAT ASSAY OF TRANSFECTED AND STIMULATED EMBRYONIC RAT
PRIMARY SKELETAL MUSCLE CULTURES**

CHLORAMPHENICOL ACETYLTRANSFERASE (CAT) ASSAY:

- Chloramphenicol solution
 - Chloramphenicol-Sigma* #C-0378
 - 0.1 M Tris-HCL pH 7.8
 - 20 mg Chloramphenicol in 50 mLs of 0.1 M Tris-HCl. Aliquot in 5 ml. volumes and
 - freeze at -20°C.
- ³H-Acetyl CoA –Amersham* # TRK 688 at 0.25 mCi/mL
- BetaMax Es – ICN # 880020
- Plastic Scintillation vials, 7 mLs – VWR* #66022-384
- Glass carrier vials – VWR* #66022-354
- Liquid Scintillation Counter

I. SOLUTIONS

- A. CHLORAMPHENICOL SOLUTION (Stock solution)
 - 20mg Chloramphenicol (Sigma* #C-0378)
 - 50mL Tris-HCL, pH 7.8
 - Aliquot in 5mL volumes and store at -20°C.
- B. ³H-ACETYL-CoA (Amersham* #TRK688, 0.25mCi/mL)
- C. CHLORAMPHENICOL: ³H-ACETYL-CoA (5mLls; 5uL)

II. PROCEDURE

1. Using the extracts from harvested cultures, combine the following in the bottom of a 7mL plastic scintillation vial (VWR* #66022-384) :
 - 50uL cell extract
 - 200uL Chloramphenicol: ³H-Acetyl-CoA
 - 1mL BetaMax ES (ICN* #8800220)
2. Put the 7 mL plastic vial into a glass carrier vial (VWR* #66022-354)

3. Count on program #5 repeatedly (at least three consecutive counts) to generate a curve and measure the slope of the diffusion of acetylated chloramphenicol in the organic phase.

From Neumann, Jr. et al. (1987) *Biotechniques* 5:5 444-447