## Acetate Incorporation Assay - FAS Activity Assay

This protocol was adapted from: Pizer, E. S., Wood, F. D., Pasternack, G. R., and Kuhajda, F. P. (1996) Cancer Res. 1996, 745–751.

**We've performed this assay on the following cell types:**

- Dissociated Primary Cortical Neurons
  - Plate at 0.5 - 1.0x$10^6$ cells/well of a 24 well plate
  - Cultures are used 7-10 days after plating.
- Dissociated Primary Hypothalamic Neurons
  - Plated at 0.4 – 0.6x$10^6$ cells/well of a 24 well plate
  - Cultures are used 7-10 days after plating.

**Drug/Compound treatment:**

- Cultures are pre-treated with the compound/drug of interest for 2 hrs @ 37°C.

**Labeling:**

- The appropriate amount of $^3$H-acetic acid to use should be determined by performing a titration to determine a concentration that falls with in the linear range of the curve. For our neuronal cultures we use a final concentration of 100 μM.
  - Add the appropriate concentration of $^3$H-acetic acid to each well and incubate cultures for an additional 2 hrs @ 37°C.
    - Acetic Acid, Sodium Salt [$^3$H]; 10.0 mCi/ml; 75-150 mCi/mmol; NEN Life Science Prod. Inc; Cat #NET-003.
  - Following the desired labeling period discard the radioactive media and wash 1X with 1 ml of PBS.

**Extraction:**

- Add 900 μl of 2:1 chloroform/methanol to each well. Add 600 μl of 4 mM $MgCl_2$ one well at a time. Triturate **2-3 times** (until homogenous) and transfer to eppi. tube.
- Vortex and spin for 1 min at high speed. Discard aqueous phase (upper) leaving a little aqueous phase to minimize error.
- Repeat extraction 3 more times with varying amounts of chloroform/methanol and $MgCl_2$:
    - 650 μl chloroform/methanol and 450 μl 4 mM and $MgCl_2$
    - 400 μl chloroform/methanol and 200 μl 4 mM and $MgCl_2$
    - 300 μl chloroform/methanol and 100 μl 4 mM and $MgCl_2$

*****It is important to remove all of the aqueous phase after the last extraction.***

- Transfer the organic phase to a scintillation vial and dry under $N_2$ for approx 5-10 min at 50-70 °C.
- Resuspend in 500 μl of 100% Ethanol then add **4 mls** of scintillation fluid and count the entire sample OR resuspend directly in scintillation fluid.