HDAC Protocol (^{3}H labeled Histone H4 Peptide)

Stock Solutions:

1. **^{3}H labeled Histone H4 Peptide:** Prepared according to above protocol; 20,000 CPM per assay is recommended.
2. **5X HDAC Assay buffer:** Add PMSF to a final concentration of 5mM prior to assay.
3. **Quenching Solution:** Add 259µl of HCl and 28µl of acetic acid to 2713µl of distilled water. Prepare immediately before use.

Assay Protocol

1. Label microcentrifuge tubes (in duplicate or as appropriate) for samples and controls (see Technical Note a).
2. To each tube add :
   - 40µl of 5X HDAC Assay Buffer stock solution with PMSF
   - 20,000 CPM [^{3}H]-acetyl Histone H 4 peptide
   - Source of HDAC (see Technical Note b)
   - distilled water to 200µl

   Prepare two control reactions. Add 10µg **HeLa Nuclear Extract** to each tube. In one tube add 10-50µl 1M **Sodium Butyrate** (see Technical Note c)
3. Centrifuge briefly to collect the components in the bottom of the tube and incubate reactions on a rocker at room temperature for up to 24 hours, or at 37°C for several hours.
4. Centrifuge briefly to collect the components in the bottom of the tube.
5. Add 50µl Quenching Solution to stop the reaction and vortex.
6. **In a chemical fume hood,** extract released [^{3}H]-acetate. Add 600µl of ethyl acetate to each tube. Vortex and centrifuge samples for one minute at 14,000 x g in a microfuge to separate phases.
7. Determine radioactivity in two 200µl aliquots of each ethyl acetate phase. Transfer each aliquot to a separate scintillation vial containing scintillation fluid, mix thoroughly and measure CPM. Compare CPM between samples incubated with or without sodium butyrate.

Technical Notes:

a. For each test sample, a second duplicate assay should be performed in the presence of 50-250mM Sodium Butyrate to demonstrate specificity of deacetylation.

b. HDAC activity is present in assayable levels in nuclear extracts and in cell lysates prepared with RIPA buffer. HDAC-containing immune complexes, washed in 1X HDAC Assay buffer may also be assayed with this protocol. Use 5µg anti-HDAC1 (Catalog # 06-720) or 10µg anti-HDAC3 (Catalog # 06-890) to immunoprecipitate the respective HDACs from 500 µg of lysate or nuclear extract.

c. For assays performed with HeLa nuclear extract, 50mM Sodium Butyrate inhibited 70-75 % of the HDAC activity.