



PRODUCTS FOR BIOLOGICAL RESEARCH  
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## Fax

To: Dr. Robertson	From: Konstantin Linnik, Ph.D.
Fax: 734 764 3562	Date: Nov. 1, 1999
Institution:	Pages: 7, including cover sheet
Department:	Re: Your inquiry: cat No.17281

Dear Dr. Robertson,

Per our phone conversation, I have attached the HDAC kit description. We use Sodium Acetate from NEN (cat # NET-003H) in our experiments. Please do not hesitate to contact our Technical Support Department if you need further information or assistance.

Sincerely,

Konstantin Linnik  
Upstate Biotechnology, Inc.  
Technical Support  
1-800-233-3991

P.S. I would like to invite you to visit our web site at [www.upstatebiotech.com](http://www.upstatebiotech.com). Available 24 hours, 7 days a week, our web site provides detailed product information and technical assistance and allows you to place orders and request literature easily and securely.



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**Histone Deacetylase Assay Kit**  
 (histone H4 peptide substrate)  
 Catalog # 17-281  
 Lot # 18895

**Kit Components**

**Histone H4 Peptide**, Catalog # 12-347, Lot # 18894, see page two for more information. Two vials, each vial containing **100µg** peptide. Store at -20°C.

**BOP Reagent**, Catalog # 20-173, Lot # 18913, see page two for more information. One vial containing **1g** of Benzotriazol-1-yloxytris (dimethylamino) phosphonium hexafluorophosphate Reagent.  $C_{12}H_{22}F_6N_8OP_2$ . Store at 4°C under nitrogen.

**Sodium Butyrate**, Catalog # 19-137, Lot # 17121, see page two for more information. One vial containing **10ml** of a 1M solution in sterile distilled water. Store at 4°C.

**HeLa Nuclear Extract**, Catalog # 12-309, Lot # 18550, see page two for more information. One vial containing **50µg** nuclear extract in 25µl. Store at -70°C.

**5X HDAC Buffer**, Catalog # 20-175, Lot # 18921. One vial containing **10ml** of 50mM Tris-HCl, pH 8.0, 750mM NaCl containing 50% glycerol. Liquid at -20°C. Store at -20°C.

**Microcon® SCX Spin Column**, Catalog # 20-174, Lot # 18920. Two vials, one vial containing two Microcon® SCX spin columns and two column support tubes; the other vial containing 8 additional column support tubes. Store at room temperature.

**FOR RESEARCH USE ONLY**  
**NOT FOR USE IN HUMANS**

**Kit Description**

**Storage:** After receipt, store individual components at temperatures recommended above and on component labels. This kit is stable for 6 months from date of shipment.

**Quantity:** Approximately 500 assays per 100µg of labeled peptide (the exact amount will vary according to labeling efficiency). Use of 20,000 CPM of [3H]-acetyl histone H4 for each assay is recommended.

**Use:** The kit contains nonradioactive components for radiolabeling and purifying Histone H4 peptide with [<sup>3</sup>H]-acetic acid. The radiolabeled peptide is a suitable substrate for assay of HDAC (histone deacetylase) activity. HeLa nuclear extract, which is rich in histone deacetylase activity, is included as a positive control. The HDAC inhibitor sodium butyrate is provided to demonstrate specificity of the deacetylase activity.

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### Additional Technical Information for Some Kit Components

#### Histone H4 Peptide (substrate for HAT activity, residues 2-20)

**Background:** This peptide corresponds to the N-terminal sequence of Histone H4 (SGRGKGGKGLGKGGAKRHRC). Once chemically acetylated with [<sup>3</sup>H]CH<sub>3</sub>COONa, the peptide may be used to measure histone deacetylase (HDAC) activity.

**Molecular weight =** 1,967.6 Daltons.

**Purity:** >90%.

**Physical Form:** Lyophilized powder.

**Storage and Stability:** Stable for 2 years at -20°C from date of shipment.

**Correction:** The label incorrectly states amino acid residues 2-19. The label should read residues 2-20.

#### BOP reagent

**Molecular weight =**442.29g

**Note:** Harmful solid reagent. Irritant. Light and moisture sensitive. Read the MSDS before use.

**Storage and Stability:** Stable for 6 months at 4°C under nitrogen gas. Light and moisture sensitive.

**Physical Form:** Powder.

#### Sodium Butyrate (Butyric Acid, sodium salt)

**Product Description:** Histone deacetylase inhibitor. Treatment of cells with sodium butyrate causes hyperacetylation of histones.

**Molecular Formula:** CH<sub>3</sub>CH<sub>2</sub>CH<sub>2</sub>COONa.

**Formula Weight:** 110.09 Dalton.

**General References:**

Miyashita, T., *et al.*, FEBS Let. **353**: 225-229, 1994.

Ohno, Y., *et al.*, Proc. Natl. Acad. Sci. USA **94**: 10279-10284, 1997.

**Purity:** > 90%

**Storage and Stability:** Stable for 1 year at 4°C from date of shipment. Liquid.

#### HeLa Nuclear Extract (positive control)

**Product Description:** Nuclear extract prepared from human HeLa cells using a modified protocol of Dignam *et al.*, which contains a variety of DNA binding proteins and transcription factors.

**Physical Form:** Frozen.

**Reference:**

Dignam, J.D., *et al.*, Nucl. Acids Res. **11**: 1475-1489, 1983.

**Storage and Stability:** Stable for 1 year at -70°C from date of shipment. For maximum recovery of product, centrifuge the original vial after thawing and prior to removing the cap. Aliquot to avoid repeated freezing and thawing.

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Other components required but not included as part of kit are:

### Reagents

- $[^3\text{H}]$ Acetic Acid (Sodium Salt): 5mCi, 2-5Ci/mmol in ethanol (NEN, Catalog # NET-003H)
- Acetonitrile (J.T. Baker, Catalog # 9017)
- Ethyl Acetate (Sigma, Catalog # E-7770)
- Triethylamine (Aldrich, Catalog #471283)
- Concentrated HCl
- Methanol
- Isopropanol
- distilled water
- scintillation fluid

### Equipment

- microcentrifuge tubes
- micropipettes and tips
- microcentrifuge
- timer
- speed-vac (optional)
- scintillation vials
- scintillation counter
- vortex mixer
- rotating wheel/platform

**Safety Warnings and Precautions:** All radioactive material is potentially dangerous, and it is important to exercise extreme caution and care when using radioisotopes. All chemicals should be considered potentially hazardous and handled with the principles of good laboratory practice.

## Quality Control Testing and Research Applications

$[^3\text{H}]$ -acetylation of Histone H4 peptide: 4.4% of  $[^3\text{H}]$ -acetate was incorporated into 100 $\mu\text{g}$  Histone H4 peptide

### HDAC Assay:

**Experiment 1:** Dependence of  $[^3\text{H}]$ -acetate Release (HDAC Activity) on the Amount of HeLa Nuclear Extract

Increasing amounts of HeLa nuclear extract were incubated overnight at room temperature with  $[^3\text{H}]$ -acetyl-Histone H4 peptide (20,000CPM). Released  $[^3\text{H}]$ -acetate was extracted with ethyl acetate and measured. HeLa nuclear extract is a source of HDAC activity.

HeLa Nuclear Extract	Mean CPM
5 $\mu\text{g}$	163
10 $\mu\text{g}$	348
20 $\mu\text{g}$	515

**Experiment 2:** Inhibition of  $[^3\text{H}]$ -acetate Release (HDAC Activity) by Sodium Butyrate

$[^3\text{H}]$ -acetyl-Histone H4 peptide was incubated over night at room temperature with 10 $\mu\text{g}$  HeLa nuclear extract in the presence or absence of Sodium Butyrate. 250mM Sodium Butyrate inhibited release of  $[^3\text{H}]$ -acetate by 94%.

HeLa Nuclear Extract	Sodium Butyrate	Mean CPM	% Inhibition
10 $\mu\text{g}$	none	265.2	-
10 $\mu\text{g}$	50mM	68.2	74
10 $\mu\text{g}$	250mM	16.2	94