

# FLUOR DE LYS®

## HDAC3/NCOR1

### fluorometric drug discovery kit

A FLUOR DE LYS® fluorescent assay system. The HDAC3/NCOR1 Fluorescent Activity Assay/Drug Discovery Kit is a complete assay system designed to measure the lysyl deacetylase activity of the recombinant human HDAC3 included in the kit. The kit is ideal for chemical library screening for candidate inhibitors or activators or kinetic assay of the enzyme under varying conditions. The preparation provided with this kit, a complex of HDAC3 with the NCOR1 Deacetylase Activation Domain (DAD), has over 100-fold greater specific activity than recombinant HDAC3 alone and more closely approximates the fully active *in vivo* form of the enzyme. Another advantage of the complex for drug discovery purposes is the possibility of identifying small molecule disruptors of the HDAC3-DAD interaction. Thus, in addition to active site binding, there is another potential route to inhibition, one more likely to be highly specific to HDAC3. The FLUOR DE LYS® HDAC3/NCOR1 assay is based on the FLUOR DE LYS® Substrate and FLUOR DE LYS® Developer combination. The assay procedure has two steps. First, the FLUOR DE LYS® SIRT1 Substrate, which comprises an acetylated lysine side chain, is incubated with HDAC3/NCOR1. Deacetylation of the substrate sensitizes the substrate so that, in the second step, treatment with the FLUOR DE LYS® Developer II produces a fluorophore.

In recent years there has been great interest in screening for HDAC inhibitors. Two pan-HDAC inhibitors, Vorinostat (SAHA) and Istodax (romidepsin), are now approved drugs for cutaneous T-cell lymphoma (CTCL). As HDAC isotype-specific inhibitors are sought, there are particularly good reasons to focus on HDAC3. Inhibition of HDAC3 may be the determining factor in the anti-proliferative effects of HDAC inhibitors on cancer cells and its caspase-dependent cleavage and relocation to the cytoplasm may be critical to the progression of apoptosis. The HDAC3/NCOR complex has been found to localize to the mitotic spindle and to be required for its proper formation and regulation. HDAC3, rather than the class II HDACs 4 and 5, is likely the direct deacetylase of MEF2, implying an important role in pathways affecting heart disease. HDAC3/NCOR1 is essential to circadian regulation of metabolism. Mice in which the NCOR1-HDAC3 interaction was disrupted had abnormal clock gene regulation and circadian behavior but were also leaner and more insulin sensitive.

- Useful for inhibitor screening or characterizing enzyme kinetics
- Includes optimal substrate selected from a panel of acetylated sites in p53 and histones
- Supplied with enough recombinant enzyme for 96 assays (1 x 96-well plate)

Citations: 8

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Ordering Information

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BML-AK531-0001

96 wells

Manuals, SDS & CofA

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# Handling & Storage

Use/Stability	Store all components, except the microplate, at -80°C for the highest stability. The HDAC3/NCOR1 Complex, Prod. No. BML-KI574, must be handled with particular care in order to retain maximum enzymatic activity. Defrost it quickly in a RT water bath or by rubbing between fingers, then immediately store on an ice bath. The remaining unused enzyme should be refrozen quickly, by placing at -80°C. If possible, snap freeze in liquid nitrogen or a dry ice/ethanol bath. To minimize the number of freeze/thaw cycles, aliquot the enzyme into separate tubes and store at -80°C.
Long Term Storage	-80°C
Shipping	Dry Ice

## Regulatory Status

RUO - Research Use Only

## Product Details

Alternative Name	Histone deacetylase 3/Nuclear receptor corepressor 1 fluorometric drug discovery kit
Application	Activity assay, Cell-based assays, Fluorescent detection, HTS

## Contents

### **HDAC3/NCOR1 Complex** (human, recombinant) (Prod. No. BML-KI574)

67 µl; 30 ng/µl in 50mM TRIS, pH 8.0, 138mM NaCl, 10% glycerol, 1 mg/ml BSA.

Storage: -80°C; avoid freeze/thaw cycles!

### **FLUOR DE LYS<sup>®</sup>-SIRT1, Deacetylase Substrate** (Prod. No. BML-KI177)

100 µl; 5mM solution in 50mM TRIS/Cl, pH 8.0, 137mM sodium chloride, 2.7mM potassium chloride, 1mM magnesium chloride.

Storage: -80°C

### **FLUOR DE LYS<sup>®</sup> Developer II Concentrate (5x)** (Prod. No. BML-KI176)

5 x 250 µl; 5x Stock Solution; dilute in assay buffer before use.

Storage: -80°C

### **Trichostatin A** (HDAC Inhibitor) (Prod. No. BML-GR309-9090)

100 µl; 0.2mM in DMSO

Storage: -80°C

### **FLUOR DE LYS<sup>®</sup> Deacetylated Standard** (Prod. No. BML-KI142)

30µl; 10mM in DMSO

Storage: -80°C

### **HDAC Assay Buffer II** (Prod. No. BML-KI422)

20 ml; (50 mM TRIS/Cl, pH 8.0, 137mM sodium chloride, 2.7mM potassium chloride, 1mM magnesium chloride, 1mg/ml BSA).

Storage: -20°C

### **HDAC Assay Buffer** (Prod. No. BML-KI143)

20 ml; (50 mM TRIS/Cl, pH 8.0, 137mM sodium chloride, 2.7mM potassium chloride, 1mM magnesium chloride).

Storage: -20°C

### **1/2 volume microplate** (Prod. No. BML-KI101)

Storage: Ambient

### **1/2 volume white NBS microplate** (Prod. No. BML-KI571)

Storage: Ambient

## UniProt ID

O15379



ENZO LIFE SCIENCES,  
INC.  
Phone: 800.942.0430  
[info-  
usa@enzolifesciences.com](mailto:info-usa@enzolifesciences.com)

European Sales Office  
ENZO LIFE SCIENCES  
(ELS) AG  
Phone: +41 61 926 8989  
[info-  
eu@enzolifesciences.com](mailto:info-eu@enzolifesciences.com)

Belgium, The Netherlands  
& Luxembourg  
Phone: +32 3 466 0420  
[info-  
be@enzolifesciences.com](mailto:info-be@enzolifesciences.com)

France  
Phone: +33 472 440 655  
[info-  
fr@enzolifesciences.com](mailto:info-fr@enzolifesciences.com)

Germany  
Phone: +49 7621 5500 526  
[info-  
de@enzolifesciences.com](mailto:info-de@enzolifesciences.com)

UK & Ireland  
Phone (UK customers):  
0845 601 1488  
Phone: +44 1392 825900  
[info-  
uk@enzolifesciences.com](mailto:info-uk@enzolifesciences.com)