FLUOR DE LYS®-Green HDAC2 fluorometric drug discovery assay kit

The HDAC2 Fluorescent Activity Assay/Drug Discovery Kit is a complete assay system designed to measure the lysyl deacetylase activity of the recombinant human HDAC2 included in the kit. The kit is ideal for chemical library screening for candidate inhibitors or kinetic assay of the enzyme under varying conditions. The FLUOR DE LYS®-Green HDAC2 assay is based on the FLUOR DE LYS®-Green Substrate and FLUOR DE LYS® Developer combination. The assay procedure has two steps. First, the FLUOR DE LYS®-Green Substrate, which comprises an acetylated lysine side chain, is incubated with HDAC2. Deacetylation of the substrate sensitizes the substrate so that, in the second step, treatment with the FLUOR DE LYS® Developer produces a fluorophore. FLUOR DE LYS®-Green is an especially sensitive substrate for HDAC2. Activity can readily be measured with enzyme amounts in the range of 1-10 ng/well (0.36 - 3.6 nM in 50 ml), thus enabling IC_{50} determinations for high affinity inhibitors. The assay is highly suitable for high-throughput screening, with a Z'-factor of 0.74.

HDAC2, like the highly homologous HDAC1, is a class I HDAC first identified as a human homolog of the yeast histone deacetylase Rpd3. HDAC2 found, along with HDAC1, in the Sin3, NuRD and CoREST, also can act independently of these complexes to deacetylate non-histone proteins such as transcription factors. In contrast to class II HDACs, which repress cardiac hypertrophy, HDAC2 activity is required for the cardiac hypertrophic response. This would suggest inhibition of HDAC2 as a possible therapeutic approach for heart disease. An opposite approach, i.e. increased HDAC2 activity or expression, might be indicated for chronic obstructive pulmonary disease (COPD), in which an HDAC2 deficit is implicated in insensitivity to glucocorticoids. The ability of the natural products theophylline and curcumin to increase HDAC2 expression is being investigated as an approach to this problem (see review). High HDAC2 expression is indicative of a poor prognosis in a number of cancers and, along with other HDACs, HDAC2 is a potential target for anti-cancer drug discovery.

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- 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)

Ordering Information

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BML-AK512-0001	96 wells

Manuals, SDS & CofA

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Handling & Storage

Use/Stability	Store all components except the microtiter plate and instruction booklet at -70°C for the highest stability. HDAC2 (Prod. No. BML-KI575) 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 -70°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 -70°C. The FLUOR DE LYS®-Green Substrate (Prod. No. BML-KI572), when diluted in Assay Buffer, may precipitate after freezing and thawing. It is best, therefore, to dilute only the amount needed to perform the assays of that day.
Long Term Storage	-80°C
Shipping	Dry Ice

Regulatory Status

RUO - Research Use Only

Product Details

Alternative Name	Histone deacetylase 2 fluorescent assay kit
Application	Activity assay, Cell-based assays, Fluorescent detection, HTS
Application Notes	Study of enzyme kinetics, modulator activity, drug discovery.

Contents	**HDAC2** (Histone Deacetylase 2) (human, recombinant) (Prod. No. BML-KI575) (67µl; 30ng/µl in 50mM TRIS, pH 8.0, 138mM NaCl, 10% glycerol, 1mg/ml BSA) Storage: -70°C. Avoid freeze/thaw cycles! **FLUOR DE LYS®-Green substrate** (Prod. No. BML-KI572) (50µl; 50mM in DMSO) Storage: -70°C **FLUOR DE LYS® Developer concentrate (20x)** (Prod. No. BML-KI105) (300µl; 20x Stock solution; Dilute in Assay Buffer before use.) Storage: -70°C **Trichostatin A** (HDAC Inhibitor) (Prod. No. BML-GR309-9090) (100µl; 0.2mM in DMSO) Storage: -70°C **FLUOR DE LYS®-Green Standard** (Prod. No. BML-KI605) (30µl 1mM in DMSO) Storage: -70°C. Note: BML-KI605 is an improved replacement of BML-KI573. **HDAC Assay Buffer II** (Prod. No. BML-KI422) (20ml; 50mM TRIS/Cl, pH 8.0, 137mM NaCl, 2.7mM KCl, 1mM $MgCl_2$, 1mg/ml BSA) Storage: -70°C **1/2 volume black NBS microplate** (Prod. No. BML-KI570) Storage: Room temperature **1/2 volume white NBS microplate** (Prod. No. BML-KI571) Storage: Room temperature
Quantity	96 assays
Technical Info / Product Notes	The original FLUOR DE LYS® HDAC Fluorescent Activity Assay revolutionized HDAC and sirtuin screening by providing a highly sensitive, mix-and-read alternative to radiolabeled, acetylated histones or peptide/HPLC methods for the assay of lysine deacetylases. The FLUOR DE LYS®-Green assay has those same advantages, but also, due to its higher wavelength excitation and emission, avoids interference by quenching or fluorescence from compounds absorbing and/or emitting in the near UV and blue. FLUOR DE LYS®-Green is an especially sensitive substrate for HDAC2.
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