ab287873 – HDAC1 IP & Activity Assay Kit

For the screening HDAC1 activity of immunoprecipitated complex/purified enzyme.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab287873

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Upon opening, use kit within 6 months.

Materials Supplied

Item	Quantity	Storage Condition
HDAC Assay Buffer	9 mL	-20°C
Cell Lysis Buffer	100 mL	-20°C
HDAC Substrate	100 µl	-20°C
Developer	500 µl	-20°C
AFC (7-amino-4-trifluoromethyl coumarin) Standard (1 mM)	100 µl	-20°C
Rabbit HDAC1 Antibody	150 µl	-20°C
Rabbit IgG (Control Antibody)	75 µl	-20°C
Protein A/G Sepharose Beads (50% slurry in 20% ethanol/H2O)	650 µl	-20°C
Positive Control (Jurkat Cell Lysate, lyophilized)	1 vial	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- 96-well white/black opaque plate with flat bottom
- Fluorescence plate reader
- Rotary mixer
- Phosphate Buffered Saline (PBS): 137 mM NaCl, 2.7 mM KCl, 10 mM Na2HPO4, 2 mM KH2PO4.
- Protease Inhibitor Cocktail
- Nuclear/Cytosol Fractionation Kit

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

HDAC Assay Buffer: Store at -20°C or 4°C. Briefly warm to 37°C before use.

Cell Lysis Buffer: Thaw cell lysis buffer and add protease inhibitors as per manufacturer's instruction. Make fresh as needed and keep on ice while in use.

HDAC Substrate: Store at -20°C. Note: Use a fresh pipette tip each time.

Developer: Aliquot 250 µl into tubes and store at -20°C. Keep on ice while in use. Use within 2 months.

AFC Standard: Store at -20°C.

Protein-A/G Sepharose Beads: Store at 4°C once you open the kit. Do not freeze.

Positive Control: Reconstitute with 25 µl deionized water. Mix gently by pipetting. Aliquot and store at -80°C. Use within 2 months.

Phosphate Buffered Saline (PBS): Chill PBS before use. Add Protease Inhibitors as per manufacturer's instruction. Make fresh as needed and keep on ice while is use.

Assay Protocol

1. **Sample preparation:**

a. **Cell Lysate:** Grow cells in 6- or 12- well plates, treat as desired. For adherent cells, remove media and wash cells with PBS. Remove the PBS, place the plate on ice and add cold lysis buffer containing protease inhibitors 125 µl/well (12-well plate) or 250 µl/well (6-well plate). Keep on ice for one minute. Scrape the cells and gently transfer the disrupted cell suspension into a pre-cooled microcentrifuge tube. Mix on a rotary mixer at 4°C for 30 minutes. Centrifuge at 10,000 g for 10 minutes at 4°C; discard cell debris pellet. For Suspension Cells, collect cells by centrifugation, wash cells with PBS at room temperature and collect cells again by centrifugation. Remove the PBS carefully and prepare cell lysates as described above for adherent cells.

b. **Nuclear Extract**: Prepare nuclear extracts from ~ 2 x 10^5 to 2 x 10^6 cells using Nuclear/Cytosol Fractionation Kit or other equivalent method.

c. **Tissue Extract**: Snap freeze dissected tissue and immediately grind to a fine powder using a mortar and pestle in a liquid N2 bath. Transfer the ground tissue to a pre-weighed chilled tube. Weigh the powder (can be stored at this point at -80°C) and add 300 µl Lysis Buffer containing protease inhibitors per 5 mg tissue. Mix on a rocker at 4˚C for about an hour. Pass the lysate through a 25-gauge needle 3X. Collect the lysate and centrifuge at high speed at 4˚C for 5 minutes to remove debris. Transfer the supernatant to a fresh tube.

d. **Protein Estimation**: Determine the protein concentration using the Bradford Assay.

Δ Note: *Repeated freeze-thaw of sample will cause loss in HDAC activity*.

2. **Immunoprecipitation:**

a. **Antibody Binding**: Cell lysates, Nuclear and Tissue extracts (CL/NE/TE) are in the linear range for HDAC IP-activity when about 50-100 µg protein content is used. If samples have high HDAC levels, you need to standardize the amount used for the IP. For each IP reaction (sample or background control), add 50-100 µg of CL/NE/TE to a pre-chilled tube on ice. Add 6 µl HDAC2 Antibody to samples [S], & 6 µl Control Antibody to the background control(s) [BC]. Bring the volume to 500 µl with PBS containing protease inhibitors. Incubate overnight at 4°C on a rotary mixer.

b. **Preparation of Protein-A/G Beads**: Use wide bore pipette tips when pipetting beads. Wash the Protein-A/G beads (25 µl of 50% slurry/reaction) 2X with 1ml PBS, centrifuge at 14000 g for ~10 seconds and aspirate the supernatant in between washes. Suspend as 50% slurry in PBS.

c. **Bead Capture**: After overnight incubation, add 25 µl of protein A/G beads slurry from step 2b to each tube and incubate for an hour at 4°C. Collect the beads by centrifugation at 14,000 g for ~10 second at 4°C. Wash beads 3X with 1ml PBS, micro-centrifuging at 14,000 g for ~10 seconds and aspirating the supernatant in between washes. Assay HDAC Activity Immediately.

3. **HDAC Activity Assay:**

a. **Reaction Mix Preparation:** Warm HDAC Assay Buffer to 37°C prior to use. Mix enough reagents for the number of assays to be performed including samples & background control(s). For each reaction, prepare 168 µl Mix containing:

	Reaction Mix
HDAC Assay Buffer	164 µl
HDAC Substrate	4 µl

Add 168 µl Reaction Mix to each sample & background control tube, mix gently and incubate at 37°C for one hour

b. **Positive Control**: In a separate tube add 2-5 µl of positive control, 4 µl HDAC Substrate, and adjust volume to 180 µl with HDAC Assay buffer. Mix gently and incubate at 37°C for one hour.

∆**Note**: The positive control is used to measure total HDAC Activity.

c. **Developer**: Add 20 µl of the developer to each tube and mix. Incubate for 30 minutes at 37°C.

d. **Standard Curve**: Dilute the AFC Standard to 10 µM by adding 10 µl of 1 mM AFC Standard to 990 µl of dH2O. Add 0, 20, 40, 60, 80, and 100 µl of diluted 10 µM AFC Standard into individual wells in a 96-well white/black plate and adjust the volume to 100 µl/well with HDAC Assay Buffer to generate 0, 200, 400, 600, 800, and 1000 pmol/well of AFC Standard respectively. Mix well.

Measurement

Centrifuge the tubes at 14,000 g for 2 minutes at room temperature. Transfer 100 µl of each reaction to individual wells in a white/black plate. Read fluorescence at Ex/Em = 380/500 nm.

Calculation:

Plot the AFC Standard Curve. Subtract the Background Control reading from all Sample readings. Apply the corrected sample reading to the AFC Standard Curve to get B pmol of AFC in the sample wells.

S**ample HDAC activity = 2 x B/TS = pmol/min/mg = mU**

Where: **B** = AFC amount from the Standard Curve (pmol)

2 = sample dilution factor**

T = reaction time (60 min)

S = sample amount (mg)

Unit Definition: One unit of HDAC is the amount of enzyme that generates one nanomole of deacetylated substrate/min/mg at 37°C. ** (Reaction Volume = 200 µl; S & BC volume = ~12 µl beads + 168 µl reaction mix + 20 µl Developer. 100µl of the reaction is used to measure the fluorescence and hence the sample dilution factor is 2).

Technical Support

For all technical or commercial enquiries please go to:

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