（FOR RESEARCH USE ONLY. DO NOT USE IT IN CLINICAL DIAGNOSIS !）

Catalog No：E-BC-F051

Specification：96T(40 samples)

Measuring instrument：Fluorescence Microplate Reader

(Ex/Em=355 nm/460 nm)

Detection range：0.019-1.027 U/L

Elabscience® Histone Deacetylase (HDAC) Activity Fluorometric Assay Kit

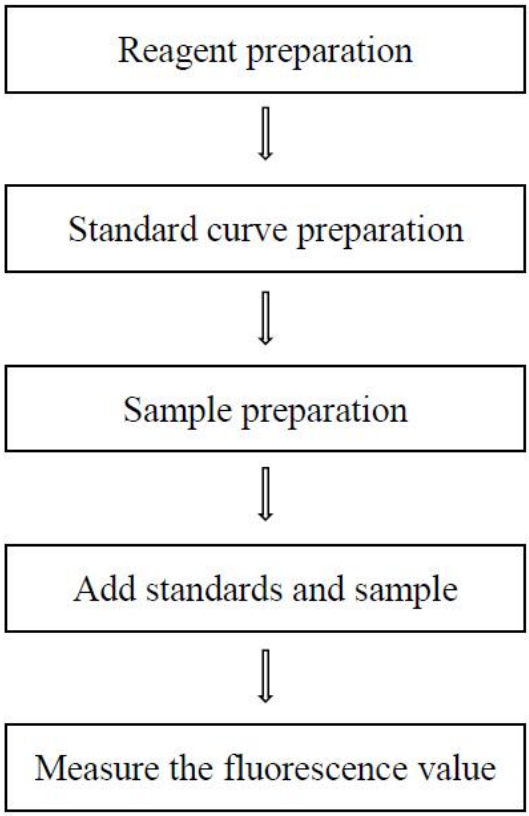
This manual must be read attentively and completely before using this product.
If you have any problem, please contact our Technical Service Center for help：

Toll-free: 1-888-852-8623
Tell: 1-832-243-6086
Fax: 1-832-243-6017
Email: techsupport@elabscience.com
Website: www.elabscience.com

Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

Table of contents

Assay summary



Intended use

This kit can be used to measure histone deacetylase (HDAC) activity in serum (plasma), cell and animal tissue samples.

Detection principle

Histone deacetylases (HDAC) are a type of protease that play an important role in the structural modification of chromosomes and the regulation of gene expression. Earlier studies have shown that HDAC has a close relationship with tumors. The overexpression of HDAC can lead to various types of cancer as well as other neurological diseases, autoimmune diseases, inflammatory diseases, heart and lung diseases.

Detection principle: HDAC specifically cleaves the fluorescent substrate, and the fluorescent group emits fluorescence which is detected at the excitation wavelength of 355 nm and the emission wavelength of 460 nm. Specific inhibitors are used in the kit to inhibit enzyme activity and eliminate the influence of non-specific enzyme activity.

Kit components & storage

Item	Component	Size (96 T)	Storage
Reagent 1	Buffer Solution	20 mL × 1 vial	-20°C, 12 months shading light
Reagent 2	Substrate	0.22 mL × 1 vial	-20°C, 12 months shading light
Reagent 3	Inhibitor	0.1 mL × 1 vial	-20°C, 12 months shading light
Reagent 4	Co-factor	1.1 mL × 2 vials	-20°C, 12 months shading light
Reagent 5	1mmol/LStandardSolution	0.4 mL × 1 vial	-20°C, 12 months shading light
	Black Microplate	96 wells	No requirement
	Plate Sealer	2 pieces	
	Sample Layout Sheet	1 piece	

Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other. For a small volume of reagents, please centrifuge before use, so as not to obtain sufficient amount of reagents.

Materials prepared by users

Instruments:

Fluorescence microplate reader (Ex/Em=355 nm/460 nm), Incubator (37°C)

Reagents:

Normal saline (0.9% NaCl)

Reagent preparation

① Equilibrate all reagents to 25℃ before use.

② The preparation of substrate working solution:
Before testing, please prepare sufficient substrate working solution according to the test wells. For example, prepare 100 μL of substrate working solution (mix well 10 μL of substrate and 90 μL of buffer solution). The substrate working solution should be prepared on spot and keep it on ice for detection. Store at -20°C for 2 days.

③ The preparation of inhibitor working solution:
Before testing, please prepare sufficient inhibitor working solution according to the test wells. For example, prepare 500 μL of inhibitor working solution (mix well 10 μL of inhibitor and 490 μL of buffer solution). The inhibitor working solution should be prepared on spot and keep it on ice for detection. Store at -20°C for 5 days.

④ The preparation of 50 μmol/L standard solution:

Before testing, please prepare sufficient 50 μmol/L standard solution. For example, prepare 2000 μL of 100 μmol/L standard solution (mix well 100 μL of 1 mmol/L standard solution and 1900 μL of buffer solution). The 50 μmol/L standard solution should be prepared on spot protected from light. The prepared solution should be used up on the same day.

⑤ The preparation of standard curve：

Always prepare a fresh set of standards. Discard working standard dilutions after use.

Dilute 50 μmol/L standard solution with buffer solution to a serial concentration. The recommended dilution gradient is as follows: 0, 5, 10, 20, 25, 30, 40, 50 μmol/L. Reference is as follows:

Item	①	②	③	④	⑤	⑥	⑦	⑧
Concentration (μmol/L)	**0**	**5**	**10**	**20**	**25**	**30**	**40**	**50**
50 μmol/L Standard (μL)	0	20	40	80	100	120	160	200
Buffer solution (μL)	200	180	160	120	100	80	40	0

Sample preparation

① Sample preparation

Plasma or serum samples: detect directly.

Tissue sample:

① Harvest the amount of tissue needed for each assay (initial recommendation 20 mg).

② Homogenize 20 mg tissue in 180 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4℃.

③ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection. Detect the prepared sample within 4 h.

④ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

Cell samples:

① Harvest the number of cells needed for each assay (initial recommendation 1×10^6 cells).

② Homogenize 1×10^6 cells in 200 μL normal saline (0.9% NaCl) with a dounce homogenizer at 4°C.

③ Centrifuge at 10000×g for 10 min at 4℃ to remove insoluble material. Collect supernatant and keep it on ice for detection. Detect the prepared sample within 4 h.

④ Meanwhile, determine the protein concentration of supernatant (E-BC-K318-M).

② **Dilution of sample**

The recommended dilution factor for different samples is as follows (for reference only):

Sample type	Dilution factor
10% Mouse liver tissue homogenate	1
10% Mouse kidney tissue homogenate	1
10% Mouse brain tissue homogenate	1
10% Mouse muscle tissue homogenate	1
10% Mouse lung tissue homogenate	1
10% Mouse spleen tissue homogenate	1
10% Mouse heart tissue homogenate	1
1×10^6 HL-60 cells	1
1×10^6 293T cells	1
1×10^6 Hela cells	1
Mouse serum	1
Human plasma	1

Note: The diluent is normal saline (0.9% NaCl). For the dilution of other sample types, please do pretest to confirm the dilution factor.

Operating steps

① Standard well: add 20 μL of standard solution with different concentrations into the wells.
Sample well: add 20 μL of sample into the wells.
Control well: add 20 μL of sample into the wells.

② Add 100 μL of buffer solution into standard and sample wells.
Add 80 μL of buffer solution into control wells.

③ Add 20 μL of substrate working solution into each well.

④ Add 20 μL of inhibitor working solution into control wells.

⑤ Incubate at 25°C for 20 min protected from light.

⑥ Add 20 μL of co-factor into each well.

⑦ Mix fully with fluorescence microplate reader for 5s. Incubate at 25°C protected from light for 20 min. Measure the fluorescence intensity at the excitation wavelength of 355 nm and the emission wavelength of 460 nm of each well.

Calculation

The standard curve:

1. Average the duplicate reading for each standard.

2. Subtract the mean F_2 value of the blank (Standard #①) from all standard readings. This is the absoluted F_2 value.

3. Plot the standard curve by using absoluted F_2 value of standard and correspondent concentration as y-axis and x-axis respectively. Create the standard curve (**y = ax + b**) with graph software (or EXCEL).

The sample:

1. Serum (plasma) sample:

Definition: The amount of enzyme in 1L serum (plasma) per 1 min that catalyze decomposition of 1 μmol product at 25°C is defined as 1 unit.

$$\text{HDAC activity(U/L)} = (\Delta F - b) \div a \div T \times f$$

2. Tissue and cell sample:

Definition: The amount of enzyme in 1 g tissue or cell protein per 1 min that catalyze decomposition of 1 μmol product at 25°C is defined as 1 unit.

$$\text{HDAC activity(U/gprot)} = (\Delta F - b) \div a \div T \times f \div C_{pr}$$

[Note]

ΔF: The absolute fluorescence value of the sample, $\Delta F = F_{sample} - F_{control}$.

f: Dilution factor of sample before tested.

T: Reaction time, 20 min.

C_{pr}: Concentration of protein in sample, gprot/L

Appendix I Performance Characteristics

1. Parameter:

Intra-assay Precision

Three human plasma were assayed in replicates of 20 to determine precision within an assay. (CV = Coefficient of Variation)

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.25	0.50	0.80
%CV	3.3	4.6	3.6

Inter-assay Precision

Three human plasma were assayed 20 times in duplicate by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (U/L)	0.25	0.50	0.80
%CV	6.9	8.8	7.5

Recovery

Take three samples of high concentration, middle concentration and low concentration to test the samples of each concentration for 6 times parallelly to get the average recovery rate of 98%.

	Sample 1	Sample 2	Sample 3
Expected Conc.(U/L)	0.25	0.50	0.80
Observed Conc.(U/L)	0.24	0.50	0.78
Recovery rate (%)	96.0	100.0	98.0

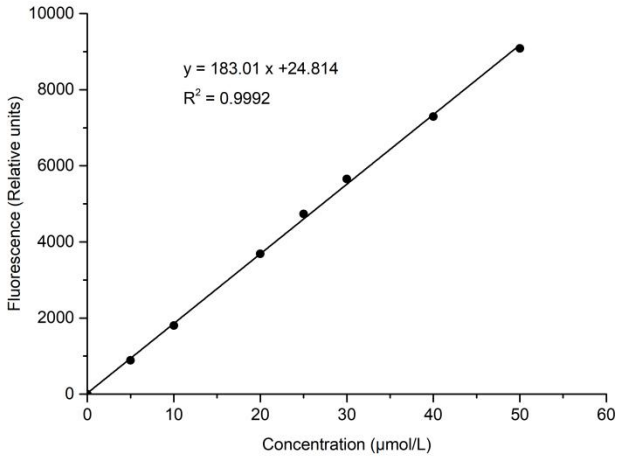
Sensitivity

The analytical sensitivity of the assay is 0.019 U/L. This was determined by adding two standard deviations to the mean O.D. obtained when the zero standard was assayed 20 times, and calculating the corresponding concentration.

2. Standard curve:

As the fluorescence value of the standard curve may vary according to the conditions of the actual assay performance (e.g. operator, pipetting technique or temperature effects), so the standard curve and data are provided as below for reference only：

Concentration (μmol/L)	0	5	10	20	25	30	40	50
Fluorescence value	3039	3908	4837	6724	7729	8696	10328	12212
	2895	3808	4699	6580	7678	8544	10192	11888
Average Fluorescence value	2967	3858	4768	6652	7704	8620	10260	12050
Absoluted Fluorescence value	0	891	1801	3685	4736	5653	7293	9083



Appendix Π Example Analysis

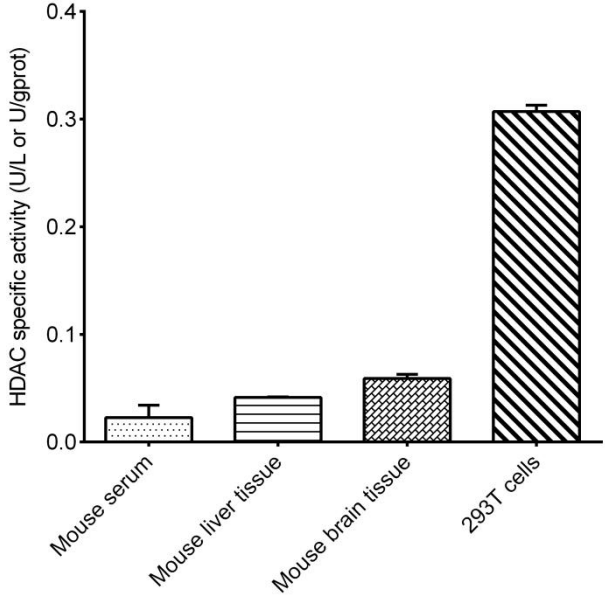
Example analysis：

Take 20 μL of 10% mouse liver tissue homogenate and carry the assay according to the operation steps. The results are as follows:

Standard curve: y = 183.01 x + 24.814, the fluorescence value of the sample well is 5012, the fluorescence value of the control well is 3098, ΔF = $F_{sample} - F_{control}$ = 5012 -3098 = 1914, the protein concentration of 10% mouse liver tissue homogenate is 12.45 gprot/L and the calculation result is:

HDAC activity (U/gprot) = (1914 - 24.814) ÷ 183.01 ÷ 20 ÷ 12.45 = 0.041 U/gprot

Detect mouse serum, 10% mouse liver tissue homogenate (the concentration of protein is 12.45 gprot/L), 10% mouse brain tissue homogenate (the concentration of protein is 3.64 gprot/L) and 1×10^6 293T cells (the concentration of protein is 0.63 gprot/L) according to the protocol, the result is as follows:


HDAC specific activity (U/L or U/gprot)
0.4
0.3
0.2
0.1
0.0
Mouse serum
Mouse liver tissue
Mouse brain tissue
293T cells

Statement

1. This assay kit is for Research Use Only. We will not response for any arising problems or legal responsibilities causing by using the kit for clinical diagnosis or other purpose.
2. Please read the instructions carefully and adjust the instruments before the experiments. Please follow the instructions strictly during the experiments.
3. Protection methods must be taken by wearing lab coat and latex gloves.
4. If the concentration of substance is not within the detection range exactly, an extra dilution or concentration should be taken for the sample.
5. It is recommended to take a pre-test if your sample is not listed in the instruction book.
6. The experimental results are closely related to the situation of reagents, operations, environment and so on. Elabscience will guarantee the quality of the kits only, and NOT be responsible for the sample consumption caused by using the assay kits. It is better to calculate the possible usage of sample and reserve sufficient samples before use.