5-HIAA (5-Hydroxyindoleacetic Acid) ELISA Kit

Article Number EKX-RKSO85-96

User Manual

For any questions regarding troubleshooting or performing the assay, please contact our support team at support@nordicbiosite.com

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General information

Size: 96T

Reactivity: Universal

Range: 1.563-100ng/ml

Sensitivity: 0.938ng/ml

Application: For quantitative detection of 5-HIAA in serum, plasma, tissue homogenates and other biological fluids.

Storage: 4°C for 6 months

Principle: Competitive

NOTE: FOR RESEARCH USE ONLY.

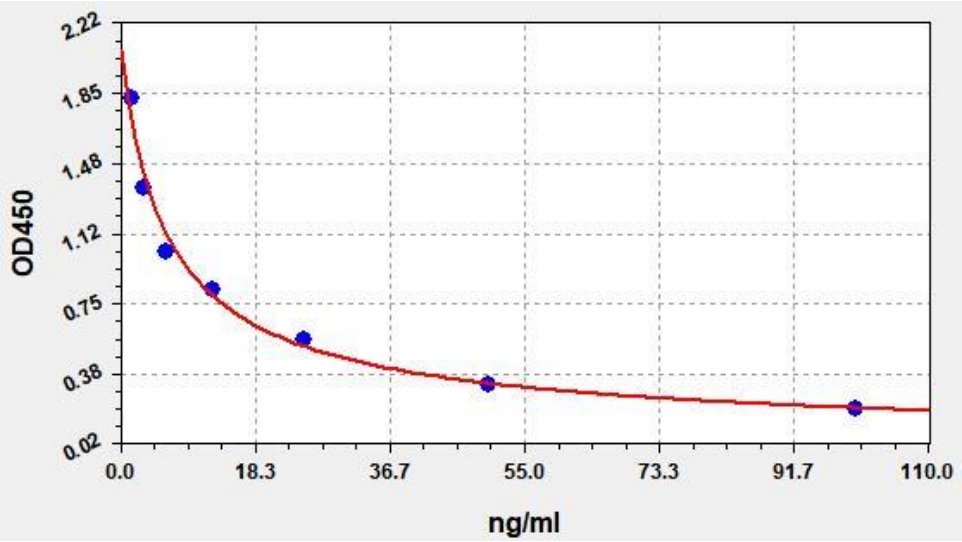
Kit Components

Item	Specifications (96T)	Storage
Micro ELISA Plate (Dismountable)	8 ×12	4℃/-20℃
Lyophilized Standard	2 vials	4℃/-20℃
Sample / Standard dilution buffer	20 ml	4℃
Biotin- labeled antibody (Lyophilized)	1 vial	2-8°C(Avoid Direct Light)
Purified water	200 µl	2-8℃
Antibody dilution buffer	10 ml	4℃
HRP-Streptavidin Conjugate (SABC)	120 µl	4℃ (Avoid Direct Light)
SABC dilution buffer	10 ml	4℃
TMB substrate	10 ml	4℃ (Avoid Direct Light)
Stop solution	10 ml	4℃
Wash buffer (25X)	30 ml	4℃
Plate Sealer	5 items	
User Manual	1 copy	

Typical Data & Standard Curve

Results of a typical standard operation of a 5-HIAA ELISA Kit are listed below. This standard curve was generated at our lab for demonstration purpose only. Users shall obtain standard curve as per experiment by themselves. (N/A=not applicable)
NOTE: For reference only.

STD (ng/ml))	OD-1	OD-2	Average
0	1.992	2.094	2.033
1.562	1.787	1.878	1.823
3.125	1.325	1.393	1.352
6.25	1.004	1.055	1.024
12.5	0.809	0.85	0.825
25	0.552	0.58	0.563
50	0.319	0.335	0.325
100	0.197	0.207	0.201



Specificity

This assay has high sensitivity and excellent specificity for detection of 5-HIAA. No significant cross-reactivity or interference between 5-HIAA and analogues was observed.

Note: Limited by current skills and knowledge, it is difficult for us to complete the cross-reactivity detection between 5-HIAA and all the analogues, therefore, cross reaction may still exist.

Recovery

Matrices listed below were spiked with certain level of 5-HIAA and the recovery rates were calculated by comparing the measured value to the expected amount of 5-HIAA in samples.

Matrix	Recovery range (%)	Average (%)
Serum (n=5)	89-99	96
EDTA plasma (n=5)	89-104	97
Heparin plasma (n=5)	87-104	98

Linearity

The linearity of the kit was assayed by testing samples spiked with appropriate concentration of 5-HIAA and their serial dilutions. The results were demonstrated by percentage of calculated concentration to the expectation.

Sample	1:2	1:4	1:8
Serum (n=5)	90-105%	89-102%	86-104%
EDTA plasma (n=5)	82-101%	84-100%	84-101%
Heparin plasma (n=5)	81-100%	93-98%	82-94%

Precision

Intra-Assay: CV<8%

Inter-Assay: CV<10%

Stability

The stability of ELISA kit is determined by the loss rate of activity. The loss rate of this kit is less than 10% within the expiration date under appropriate storage condition.

Standard (n=5)	37°C for 1 months	4°C for 6 months
Average (%)	80	95-100

To minimize extra influence on the performance, operation procedures and lab conditions, especially room temperature, air humidity, incubator temperature should be strictly controlled. It is also strongly suggested that the whole assay is performed by the same person from the beginning to the end.

Principle of the Assay

This kit was based on Competitive-ELISA detection method. The microtiter plate provided in this kit has been pre-coated with target. During the experiment, target in the sample or standard competes with a fixed amount of target on the solid phase supporter for sites on the Biotinylated Detection Antibody specific to target. Excess conjugate and unbound sample or standard are washed from the plate. HRP-Streptavidin (SABC) is added to each microplate well and incubated. Then TMB substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm. The concentration of target in the samples is then determined by comparing the OD of the samples to the standard curve.

Precautions for Use

To inspect the validity of experiment and to ensure the correct sample dilution, a pilot experiment using standards and a small number of samples is recommended.
After opening and before using, keep the plate dry.
Before using the kit, spin the tubes to bring down all components to the bottom of tubes.
Store TMB reagents protected from light.
Washing process is very important. Incomplete wash easily cause a false positive result and high background.
Duplicate well assay is recommended for both standard and sample testing.
Do not let microplate dry during the assay. A dry plate will inactivate active components on plate.
Do not reuse tips and tubes to avoid cross contamination.
Avoid using reagents from different batches together.

Material Required But Not Supplied

1. Microplate reader (wavelength: 450 nm)
2. 37℃ incubator
3. Automated plate washer
4. Precision single and multi-channel pipette and disposable tips
5. Clean tubes and Eppendorf tubes
6. Deionized or distilled water

Washing

Manual: Discard the solution in the plate without touching the side walls. Gently tap the plate on absorbent filter papers or other absorbent material. Fill each well completely with 350µl wash buffer and soak for 1 to 2 min, then aspirate contents from the plate, and tap again the plate on absorbent filter papers or other absorbent material.

Automatic: Aspirate all wells, and then wash plate with 350µl wash buffer. After the final wash, invert the plate, and gently tap the plate on absorbent filter papers or other absorbent material. It is recommended that the washer is set for soaking 1 minute. (Note: set the height of the needles; be sure the fluid can be sipped up completely)

Sample Collection and Storage (universal)

- Serum: Place whole blood sample at room temperature for 2 hours or leave it at 4°C overnight and centrifuge for 20 min at approximately 1000×g. Collect the supernatant and carry out the assay immediately. Blood collection tubes should be disposable, non-pyrogenic, and non-endotoxin.
- Plasma: Collect plasma using (EDTA-Na_2 or heparin as an anticoagulant. Centrifuge samples for 15 minutes at 1000×g at 2 - 8°C within 30 minutes of collection. Collect the supernatant and carry out the assay immediately. Avoid hemolysis and high cholesterol samples.
- Tissue Homogenates: As hemolysis affects the result of the assay, it is necessary to remove residual blood by washing tissue with pre-cooled PBS buffer (0.01M, pH=7.4). Mince tissue after weighing it and homogenized it in PBS (the volume depends on the weight of the tissue). Normally, 9mL PBS would be appropriate for 1g of tissue sample. It is recommended to add protease inhibitors into the PBS) with a glass homogenizer on ice. To further break the cells, we recommend sonicating the suspension with an ultrasonic cell disrupter or subject it to freeze-thaw cycles. The homogenates must then be centrifuged for 5min at 5000×g to get the supernatant. The total protein concentration is determined by a BCA assay and the total protein concentration in each well should not exceed 0.3mg.
- Cell Culture Supernatant: Centrifuge supernatant for 20 minutes at 1000×g at 2 - 8°C to remove insoluble impurity and cell debris. Collect the clear supernatant and carry out the assay immediately.

- Cell Culture Lysate: Commercial RIPA kits are recommended. Follow the provided instructions. Generally, 0.5ml RIPA lysis buffer would be appropriate to $2x10^6$cells, DNA must be removed. The total protein concentration is determined by a BCA assay and the total protein concentration in each well should not exceed 0.3mg.
- Other Biological Fluids: Centrifuge samples for 20 min at 1000×g at 2-8°C. Collect supernatant and carry out the assay immediately.

Note: Samples to be used within 5 days can be stored at 4°C, besides that, samples must be stored at -20°C (assay ≤1 month) or -80°C (assay≤2 months) to avoid loss of bioactivity and contamination. Avoid multiple freeze-thaw cycles. Hemolytic samples are not suitable for this assay.

Sample Dilution Guideline

The user should estimate the concentration of target protein in the test sample and select a proper dilution factor to make the diluted target protein concentration fall in the optimal detection range of the kit. Dilute the sample with the provided dilution buffer. Several trials may be necessary. The test sample must be well mixed with the dilution buffer. Both standard curves and sample should be included in pilot experiment. In case of samples with very high concentrations, dilute the samples with PBS first and then dilute the samples with Sample Dilution.

Reagent Preparation and Storage

Bring all reagents to room temperature for 20 min before use.

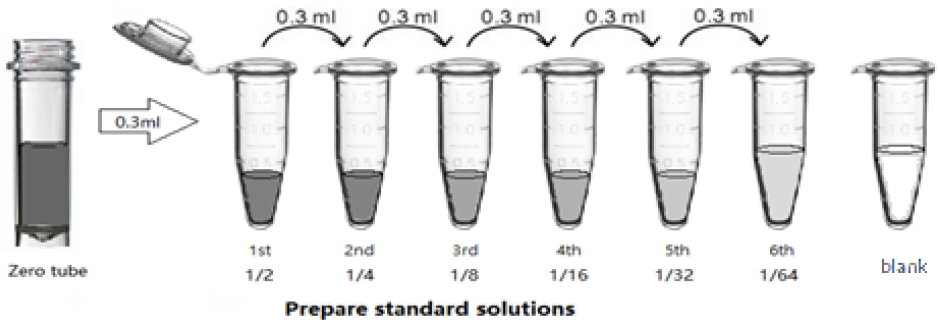
1. Wash Buffer

If crystals have formed in the concentrate, warm it in 40°C water bath (heating temperature should not exceed 50°C) and mix it gently until the crystals have completely been dissolved. The solution should be cooled to room temperature before use.
Dilute 30ml Concentrated Wash Buffer into 750ml Wash Buffer with deionized or distilled water. Place the rest of the solution at 4°C.

2. Standard

1). Add 1 ml Sample Dilution Buffer into one Standard tube (labeled as zero tube), keep the tube at room temperature for 10 minutes and mix them thoroughly.
Note: If the standard tube concentration is higher than the range of the kit, please dilute it and label as zero tube.

2). Label 7 EP tubes with 1/2, 1/4, 1/8, 1/16, 1/32, 1/64 and blank respectively. Add 0.3ml of the Sample Dilution Buffer into each tube. Add 0.3ml of the above Standard solution (from zero tube) into 1st tube and mix thoroughly. Transfer 0.3ml from 1st tube to 2nd tube and mix thoroughly. Transfer 0.3ml from 2nd tube to 3rd tube and mix thoroughly, and so on. Sample Dilution Buffer was used for the blank control.



Note: It is recommended to use Standard Solutions within 2 hours.

3. Preparation of Biotin-detection Antibody working solution

Prepare it within 1 hour before experiment.

1) Dissolve: Add 70ul purified water into tube and mix them thoroughly, after the biotin-labelled antibody is dissolved, please store it at 2-8°C.

2) Calculate required total volume of the working solution: 50ul/well x quantity of wells. Allow 0.1-0.2 ml more than the total volume.

3) Dilute the Biotin-detection antibody with Antibody Dilution Buffer at 1:100 and mix them thoroughly. (i.e. Add 1μl Biotin-labelled antibody into 99μl Antibody Dilution Buffer.)

4. Preparation of HRP-Streptavidin Conjugate (SABC) Working Solution:

Prepare it within 30min before experiment.

1) Calculate required total volume of the working solution: 0.1ml / well × quantity of wells. (Allow 0.1-0.2ml more than the total volume)

2) Dilute the SABC with SABC Dilution Buffer at 1:100 and mix thoroughly. (i.e. Add 1μl of SABC into 99μl of SABC Dilution Buffer.)

Assay Procedure

When diluting samples and reagents, they must be mixed completely and evenly. Before adding TMB into wells, equilibrate TMB Substrate for 30 min at 37 °C. It is recommended to plot a standard curve for each test.

1.Set standard, test samples, control (blank) wells on the pre-coated plate respectively, and then record their positions. It is recommended to measure each standard and sample in duplicate.

2. Add Sample and Biotin- labeled Antibody: Add 50µl of Standard, Blank, or Sample per well. The blank well is Sample/Standard Dilution Buffer. Immediately add 50µL Biotin-labeled Antibody Working Solution into each well. Cover with the Plate sealer we provided. Gently tap the plate to ensure thorough mixing. Incubate for 45minutes at 37°C (we recommend to add solutions to the bottom of microplate well. Avoid touching the inner wall and foaming as much as you can.)
3.Wash: Remove the cover and wash the plate 3 times with Wash Buffer. Let the wash buffer stay in the wells for 1 minute each time. After the last wash, remove any remaining Wash Buffer by aspirating or decanting.

4.HRP-Streptavidin Conjugate (SABC): Add 100µl SABC Working Solution into each well. Cover it with a new Plate sealer. Incubate for 30 minutes at 37°C.

5.Wash: Remove the cover and wash plate 5 times with Wash Buffer and let the wash buffer stay in the wells for 1-2 min each time.

6.TMB Substrate: Add 90µl TMB Substrate into each well, cover the plate and incubate at 37°Cin dark for 10-20 min. (Note: The reaction time can be shortened or extended according to the actual color change, but not more than 30 min. You can terminate the reaction when apparent gradient appears in standard wells.)

7.Stop: Add 50µl Stop Solution into each well. The color will turn yellow immediately. The adding order of Stop Solution should be the same as the TMB Substrate Solution.

8.OD Measurement: Read the O.D. absorbance at 450 nm in Microplate Reader immediately after adding the stop solution.
Regarding calculation, the standard curve is plotted as the O.D.450 of each standard solution (Y) versus the respective concentration of the standard

solution (X). The target concentration of the samples is later interpolated from the standard curve. It is recommended to use some professional software to do this calculation, such as Curve Expert 1.3 or 1.4.
Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from the interpolation to obtain the concentration before dilution.

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