Elabscience®

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DZP (Diazepam) ELISA Kit

Catalog No: E-FS-E027

96T

This manual must be read attentively and completely before using this product.

If you have any problems, please contact our Technical Service Center for help.

Phone: 240-252-7368(USA) 240-252-7376(USA)

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.

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Test principle

This kit uses Indirect-Competitive-ELISA as the method. It can detect DZP (Diazepam) in urine, tissues and feed. This kit is composed of Micro ELISA Plate pre-coated with coupled antigen, HRP conjugate, antibody, standard and other supplementary reagents. During the detection, after adding standard or sample solution, DZP in the samples competes with pre-coated coupled antigen on the Micro ELISA Plate for DZP antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each micro plate well, and TMB substrate is added for color development. There is a negative correlation between the OD value of samples and the concentration of DZP. The residual quantity of DZP in the samples can be calculated by comparing the OD of the samples to the standard curve.

Technical indicator

Sensitivity: 0.3 ppb (ng/mL)

Reaction mode: 25℃, 30 min~30 min~15 min

Detection limit: Urine ---5 ppb, Tissue (chicken, beef, pork) ---5 ppb,
Compound feed ---50 ppb, Condensed feed/ Premix feed---100 ppb.

Cross-reactivity: Diazepam ---100%, Nitrazepam---<10%, Oxazepam---<10%.

Sample recovery rate: Tissue ---90%±20%.

Kits components

Item	Specifications
Micro ELISA Plate	96 wells
Standard Liquid (black cap)	1mL each (0 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb, 24.3 ppb)
High Concentrated Standard (1 ppm)	1 mL
HRP Conjugate (red cap)	11 mL
Antibody Working Solution(blue cap)	5.5 mL
Substrate Reagent A(white cap)	6 mL
Substrate Reagent B(black cap)	6 mL
Stop Solution(yellow cap)	6 mL
20×Concentrated Wash Buffer (white cap)	40 mL
2×Reconstitution Buffer (yellow cap)	50 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

Other supplies required

Instruments: Micro-plate reader, Printer, Homogenizer, Nitrogen Evaporators, Oscillators, Centrifuge, Graduated pipette, Balance (sensibility 0.01 g).

High-precision transferpettor: Single channel (20-200 μL, 100-1000 μL), Multichannel (300 μL).

Reagents: NaOH, N-hexane.

Experimental preparation

1. Sample pretreatment Notice:

Experimental apparatus should be clean and the pipette should be disposable to avoid cross-contamination during the experiment.

2. Reagent preparation

Solution 1: Reconstitution Buffer

Dilute the 2×Reconstitution Buffer with deionized water. (2×Reconstitution Buffer: Deionized water=1:1) .The Reconstitution buffer can be store at 4℃ for a month.

Solution 2: Wash Buffer

Dilute the 20× Concentrated Wash Buffer with deionized water 20× Concentrated Wash Buffer: Deionized water=1:19).

Solution 3: 0.1 M NaOH Solution

Dissolve 4 g NaOH with 1000 mL deionized water.

Solution 4: Wash Buffer

Dilute 20×Concentrated Wash Buffer with deionized water. (20×Concentrated Wash Buffer (V): Deionized water (V) = 1:19)

3. Sample pretreatment procedure

3.1 Pretreatment of tissue

(1) Weigh 2±0.05 g of crushed homogenate tissue sample, add 8 mL of 0.1 M NaOH Solution. Oscillate fully for 5 min, centrifuge at a speed of over 4000 r/min for 10 min at room temperature.

(2) Take 1 mL of the supernatant, add 10 mL of N-hexane. Oscillate fully for 5 min, centrifuge at a speed of over 4000 r/min for 5 min at room temperature.

(3) Take 1 mL of the upper N-hexane phase and blow it dry.

(4) Take 1 mL of the Reconstitution buffer to redissolve the sediment. Take 50 μL for analysis.

Note: Sample dilution factor: 10, minimum detection dose: 5 ppb

3.2 Pretreatment of urine sample:

(1) Take 1 mL of clear urine sample into 50 mL centrifuge tube. Add 4 mL of 0.1 M NaOH Solution. Oscillate fully for 2 min.

(2) Take 1 mL of the mixture, add 10 mL of N-hexane. Oscillate fully for 5 min, centrifuge at a speed of over 4000 r/min for 5 min at room temperature.

(3) Take 1 mL of the upper N-hexane phase and blow it dry.

(4) Take 1 mL of the 1×Reconstitution solution to redissolve the sediment. Take 50 μL for analysis.
Note: Sample dilution factor: 10, minimum detection dose: 5 ppb.

3.3 Pretreatment of fodder sample:

(1) Weigh 1±0.05 g of homogenate fodder sample, add 1 mL of deionized water and 3 mL of 0.1 M NaOH Solution. Oscillate fully for 2 min.
(2) Add 10 mL of N-hexane. Oscillate fully for 10 min, centrifuge at a speed of over 4000 r/min for 10 min at room temperature.
(3) Take 1 mL of the upper N-hexane phase and blow it dry.
(4) Take 1 mL of the Reconstitution buffer to redissolve the sediment. Then dilute it with the following ratio.
For compound feed sample: Dilute the Sample extract with Reconstitution buffer for 10 times (Sample extract: Reconstitution buffer = 1:9).
Note: Sample dilution factor: 100, minimum detection dose: 50 ppb
For condensed feed/ premix feed sample: Dilute the Sample extract with Reconstitution buffer for 20 times (Sample extract: Reconstitution buffer = 1:19).
Note: Sample dilution factor: 200, minimum detection dose: 100 ppb

Assay procedure

Centrifuge the sample again after thawing before the assay. Bring all reagents to room temperature before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming.

1. **Number:** Number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells.
2. **Add sample:** Add 50 μL of Standard or Sample per well, then add 50 μL antibody working solution, cover the plate with sealer. Oscillate for 5 sec gently to mix thoroughly. Incubate for 30 min at 25℃ in the dark.
3. **Wash:** Uncover the sealer carefully, remove the liquid in each well. Immediately add 250 μL of wash buffer to each well and wash. Repeat wash procedure for 5 times, 30 sec intervals/time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
4. **HRP Conjugate:** Add 100 μL of HRP conjugate to each well. Incubate for 30 min at 25℃ in the dark.
5. **Wash:** Repeat Step 3.
6. **Color Development:** Add 50 μL of substrate solution A to each well, and then add 50 μL of substrate solution B. Gently oscillate for 5 sec to mix thoroughly. Incubate for 15 min at 25℃ in the dark.
7. **Stop reaction:** Add 50 μL of stop solution to each well, gently oscillate for 5 sec.
8. **OD Measurement:** Determine the optical density (OD value) of each well at 450 nm with a microplate reader (the 450/630 nm double wavelength is recommended). This step should be finished in 10 min after stop reaction.

Result analysis

1. **Absorbance% =A/A_0×100%**

   A: Average absorbance of standard solution or sample

   A_0: Average absorbance of 0 ppb Standard solution

2. **Drawing and calculation of standard curve**

   Create a standard curve by plotting the absorbance percentage of each standard on the y-axis against the log concentration on the x-axis to draw a semi-logarithmic plot. Add the average absorbance value of sample to standard curve to get corresponding concentration. If samples have been diluted, the concentration calculated from the standard curve must be multiplied by the dilution factor.

   For this kit, it is more convenient to use professional analysis software for accurate and fast analysis on many samples.

Notes

1. Overall OD value will be lower when reagents have not been brought to room temperature before use or room temperature is below 25℃.
2. During the washing procedure, if the wells turn dry, it will lead to bad linear standard curve and poor repeatability, move on to the next step immediately after wash.
3. Mix thoroughly and wash the plate completely. The consistency of wash procedure can strongly affect the reproducibility of this ELISA kit.
4. Micro ELISA plate should be covered by plate sealer. Avoid the reagents to strong light.
5. Do not use expired kit and reagents of different batches.
6. TMB should be abandoned if it turns color. When OD value of standard(concentration: 0)＜0.5 unit($A_{450\ nm}$＜0.5), it indicates the reagent may be deteriorated.
7. Stop solution is caustic, avoid contact with skin and eyes.

Storage and valid period

Storage: Store at 2-8℃. Avoid freeze / thaw cycles.

Valid Period: 1 year, production date is on the packing box.

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