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Diazepam ELISA Kit

10/19

(Catalog # E4772-100; 96 assays, Storage at 4°C)

I. Introduction:

Diazepam is mainly used to treat anxiety, insomnia, panic attacks and symptoms of acute alcohol withdrawal. It is also used as a premedication for inducing sedation, anxiolysis, or amnesia before certain medical procedures (e.g., endoscopy). Diazepam is the drug of choice for treating benzodiazepine dependence with its long half-life allowing easier dose reduction. It is frequently employed in animal production for its excellent growth promotion properties. However, in humans, an overdose of diazepam leads to severe side effects. This has led to a prohibition of diazepam for the treatment of animals used for food production. BioVision's Diazepam ELISA Kit is based on Competitive ELISA method. It can detect Diazepam in Tissue, Urine and Feed samples. The microtiter plate provided in the kit has been pre-coated with Diazepam. During the reaction, Diazepam in the samples or standard competes with coated Diazepam for anti- Diazepam antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each microtiter 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 Diazepam. The concentration of Diazepam in the samples can be calculated by comparing the OD of the samples to the standard curve.

II. Applications:

In vitro, quantitative determination of Diazepam

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

Sensitivity: 0.3 ppb (ng/mL)

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

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

III. Sample Type:

Tissue, Urine, Feed

IV. Kit Contents:

Components	E4772-100	Part Number
Micro ELISA Plate	96 wells	E4772-100-1
Standard	6 X 1 ml	E4772-100-2
HRP Conjugate	5.5 ml	E4772-100-3
Antibody Working Solution	5.5 ml	E4772-100-4
Substrate Reagent A	6 ml	E4772-100-5
Substrate Reagent B	6 ml	E4772-100-6
Stop Solution	6 ml	E4772-100-7
Wash Buffer (20X)	40 ml	E4772-100-8
Reconstitution Buffer (2X)	50 ml	E4772-100-9
Plate Sealer	3	E4772-100-10

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- 0.1 M NaOH, N-hexane
- Clean Eppendorf tubes for preparing standards or sample dilutions

VI. Storage and Handling:

Store at 4ºC**.**

VII. Reagent and Sample Preparation:

Bring all reagents to room temperature before use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.

- **Wash Buffer (20X):** Dilute 20X Concentrated Wash Buffer to 1X with deionized water.
- **Reconstitution Buffer (2X):** Dilute 2X Reconstitution Buffer with deionized water. Mix 10x Reconstitution Buffer (V): Deionized water (V) =1:1). The Reconstitution buffer can be store at 4℃ for a month.
- **0.1 M NaOH** : Dissolve 0.4 g NaOH with 100 ml deionized water
- **Standard:**

Standard	S1	S2	S3	S4	S5	S6
Concentration (ppb)	0	0.3	0.9	2.7	8.1	24.3

VIII. Sample Preparation:

Sample pretreatment:

- **Pretreatment of tissue :**
  Weigh 2 ± 0.05 g of crushed homogenate tissue sample, add 8 mL of 0.1 M NaOH Solution. Mex well for 5 min, centrifuge at a speed of over 4000 r/min for 10 min at room temperature. Take 1 ml of the supernatant; add 10 ml of N-hexane. Mix well for 5 min, centrifuge at a speed of over 4000 r/min for 5 min at room temperature. Take 5 ml of the upper N-hexane phase and blow it dry. 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.

- **Pretreatment of urine:**
  Take 1 mL of clear urine sample into 50 ml centrifuge tube. Add 4 ml of 0.1 M NaOH Solution. Mix well for 2 min. Take 1 ml of the mixture; add 10 ml of N-hexane. Mix well for 5 min, centrifuge at a speed of over 4000 r/min for 5 min at room temperature. Take 5 ml of the upper N-hexane phase and blow it dry. Take 1 ml of the 1x Reconstitution solution to redissolve the sediment. Take 50 µl for analysis.
  Note: Sample dilution factor: 10, minimum detection dose: 5 ppb.

- **Pretreatment of feed sample:**
  Weigh 1± 0.05 g of homogenate feed sample, add 1 ml of deionized water and 3 ml of 0.1 M NaOH Solution. Mix well for 2 min. Add 10 mL of N-hexane. Mix well for 10 min, centrifuge at a speed of over 4000 r/min for 10 min at room temperature. Take 1 ml of the upper N-hexane phase and blow it dry. 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

IX. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay. It is recommended that all standards and samples be run at least in duplicate. A standard curve must be run with each assay.

1. Add 50 µl of each **standard or samples** into appropriate wells.
2. Add 50 µl of **Antibody Working Solution**. Cover the plate with the sealer provided in the kit. Gently mix and incubate for 30 min. at 25°C.
3. Aspirate the solution from each well add 300 µl of **1x wash buffer** to each well. Leave it for 30 sec, aspirate the solution from each well and pat it dry against clean absorbent paper. Repeat this wash step 5 times.
4. Add 100 µl **HRP Conjugate** to each well. Incubate for 30 min at 25°C in the dark.
5. Repeat wash Step 3.
6. Add 50 µl of **Substrate Reagent A** to each well and then add 50 µl of **Substrate Reagent B.** Cover with a new plate sealer. Incubate for about 15 min at 25°C. Protect the plate from light. Note: the reaction time can be shortened or extended according to the actual color change, but not more than 30 min.
7. Add 50 µl of **Stop Solution** to each well. Note: adding the stop solution should be done in the same order as the substrate solution.
8. Read the absorbance in micro plate reader set to 450 nm reference wavelength 630 nm. This step should be performed within 5 min after stop reaction.

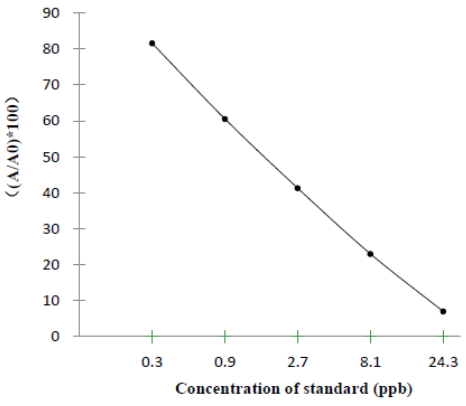
X. Calculation:

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 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.

Absorbance (%)=A/A_0 ×100%

A: Average absorbance of standard or sample

A_0 : Average absorbance of 0 ppb Standard



Typical standard curve and data is provided below for reference only. A standard curve must be run with each assay

Concentration of standard (ppb)	OD-1	OD-2	Average OD
0	2.6181	2.7355	2.6768
0.3	2.1399	2.2216	2.1808
0.9	1.6146	1.6229	1.6188
2.7	1.1212	1.0859	1.1036
8.1	0.6136	0.6156	0.6146
24.3	0.1900	0.1825	0.1863

XI. RELATED PRODUCTS:

- Enrofloxacin (ENR) ELISA Kit (E4277)
- Chloramphenicol (CAP) ELISA Kit (K4230)
- Cimaterol ELISA Kit (E4771)
- Sulfamethoxazole ELISA Kit (E4770)

FOR RESEARCH USE ONLY! Not to be used on humans.