QUALICHEK™ Diazepam ELISA

REF : KBFP1020

Ver 1.0

RUO

Quantitative testing of Diazepam in samples, such as urine, tissues and feed, etc.

RUO	**For Research Use Only**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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 **KRISHGEN BioSystems|** For US / Europe: toll free +1(888)-970-0827 tel: +1(562)-568-5005
For Asia / India: tel: +91(22)-49198700
Email: sales@krishgen.com

Introduction:

Diazepam, first marketed as Valium, is a medicine of the benzodiazepine family that typically produces a calming effect. It is commonly used to treat a range of conditions including anxiety, alcohol withdrawal syndrome, benzodiazepine withdrawal syndrome, muscle spasms, seizures, trouble sleeping, and restless legs syndrome. It may also be used to cause memory loss during certain medical procedures.

Intended Use:

This kit is for quantitative testing for Diazepam in sample, such as urine, tissues, feed, etc.

Principle:

This kit uses Indirect-Competitive-ELISA as the method. It can detect DZP in samples, such as urine, tissues and feed etc. This kit is composed of ELISA Microtiter plate, HRP conjugate, antibody, standard and other supplementary reagents. The microplate provided in this kit has been pre-coated with coupled antigen. During the reaction, DZP in the samples or standard competes with coupled antigen on the solid phase supporter for sites of DZP antibody. Then Horseradish Peroxidase (HRP) conjugate is added to each micro plate well, and TMB substrate is for color development. There is a negative correlation between the OD value of samples and the concentration of DZP. The concentration of DZP in the samples can be calculated by comparing the OD of the samples to the standard curve.

Materials Provided:

1. Coated Microtiter plate - 1x 96 wells
2. Standards Liquid -1mL each (0 ppb, 0.3 ppb, 0.9 ppb, 2.7 ppb, 8.1 ppb, 24.3 ppb)
3. High Concentrated Standard (1 ppm) - 1mL
4. HRP Conjugate - 11 mL
5. Antibody Working Solution - 5.5 mL
6. Substrate A - 6 mL
7. Substrate B - 6 mL
8. Stop Solution - 6 mL
9. Wash Buffer (20X) - 40 mL
10. Plate Sealer - 3 pieces

Material required but not provided:

Instrument: Microtiter plate reader, Printer, Homogenizer, Nitrogen Evaporators/Water bath, Oscillators, centrifuge, Graduated pipette, Balance (sensibility 0.01 g)
High-precision transferpettor:single channel (20-200 µL, 100-1000 µL), Multichannel (300 µL) micropipettes.
Reagents: NaOH, N-hexane.

Storage Information:

1. Store main kit components at 2-8°C Avoid freeze / thaw cycles.
2. Before using, bring all components to room temperature (18-25°C). Upon assay completion return all components to appropriate storage conditions

Sample pretreatment:

Bring all reagents and samples to room temperature before use.

Open the microplate reader in advance, preheat the instrument, and set the testing parameters.

1. Sample pretreatment Notice:

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

2. Solution 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.

3. Sample pretreatment procedure:

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 roomtemperature.
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 5 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

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 5 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.

Pretreatment of feed sample:
1. Weigh 1±0.05 g of homogenate feed 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 for 30 min 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 of antibody working solution, cover the plate with sealer, oscillate for 5 s gently to mix thoroughly, incubate for 30 min at 25℃.
3. **Wash:** uncover the sealer carefully; remove the liquid in each well. Immediately add 300 µL of wash buffer to each well and wash. Repeat wash procedure for 5 times, 30 s 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 100µl of HRP conjugate to each well, incubate shading light for 30 min at 25^0C
5. **Wash**: repeat step 3.
6. **Color Development:** add 50 µL of Substrate Reagent A to each well, and then add 50 µL of Substrate Reagent B. Gently oscillate for 5 s to mix thoroughly. Incubate shading light for 15 min at 25℃ (The reaction time may be shortened or prolonged according to the depth of the color).
7. **Stop reaction:** add 50 µL of stop solution to each well, oscillate gently to mix thoroughly.
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.

Judgment of result:

Absorbance (%) =A/A0×100%

A: Average absorbance of standard or sample

A_0: Average absorbance of 0 ppb Standard

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 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 a large number of samples.

Notes:

1. The overall OD value will be lower when reagents have not been brought to room temperature before use or room temperature is below 25℃.
2. If the wells turn dry during the washing procedure, it will lead to bad linear standard curve and poor repeatability. Operate 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. ELISA Microtiter plate should be covered by plate sealer. Avoid the reagents to strong light.
5. Do not use expired kit or reagents of different batches.
6. TMB should be abandoned if it turns color. When OD value of standard (concentration: 0)＜0.5 unit (A450nm＜0.5), it indicates the reagent may be deteriorated.
7. Stop solution is caustic; avoid contact with skin and eyes.

Technical specification:

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.
Reactivity: Diazepam ---100%, Nitrazepam---<10%, Oxazepam---<10%.
Sample recovery rate: Tissue ---90%±20%.

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