Version 26-11/24

**User's Manual**

# Xylazine ELISA Kit

REF **DEIA-BY053**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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# PRODUCT INFORMATION

## Intended Use

This kit is a competitive enzyme-labeled immunoassay and can be used in quantitative analysis of xylazine in serum and plasma.

## Principles of Testing

This ELISA kit is designed to detect xylazine based on the principle of "indirect- competitive" enzyme immunoassay. The microtiter wells are coated with BSA-linked antigen. Xylazine in the sample competes with antigen coated on the microtiter plate for the antibody. After the addition of enzyme conjugate, chromogenic substrate is used, and the signal is measured by spectrophotometer. The absorption is inversely proportional to the xylazine concentration in the sample.

## Reagents And Materials Provided

1. Microtiter plate with 96 wells coated with antigen
2. Standard solutions×6 bottles: 1mL/bottle. 0ng/mL, 0.37ng/mL, 1.1ng/mL, 3.3ng/mL, 10ng/mL, 30ng/mL
3. Antibody solution, 7mL
4. Enzyme conjugate, 12mL
5. Substrate solution , 6mL×2
6. Stop solution, 7mL
7. 20×Wash solution, 50mL
8. Sample diluent, 2×50mL

## Materials Required But Not Supplied

1. Microplate reader (wavelength:450nm/620nm)
2. Polystyrene centrifuge tubes
3. Precision single and multi-channel pipette and disposable tips
4. Paper towels or equivalent absorbent material
5. Timer
6. Deionized or distilled water
7. Incubator

## Storage

Store the test kit and components at 2-8°C. The unopened reagents are stable at 2-8°C until the indicated expiry date.

## Specimen Collection And Preparation

Serum: To collect serum, use a serum separator tube and allow the whole blood to clot for 25-35 minutes. Then centrifuge blood for 5 minutes at 3000 x g and remove serum immediately.

Plasma: To collect plasma, use EDTA, heparin, or citrate as an anticoagulant. Within 30 minutes of whole blood collection, centrifuge for 15 minutes at 1500 x g and remove plasma immediately.

Allow samples to reach room temperature prior to assay. Take care to agitate patient samples gently to ensure homogeneity.

Samples must be diluted at least 1 in 40 (v/v), e.g. 10 µl sample in 390 µl sample diluent, prior to assay.

## Reagent Preparation

### 1× Wash solution

Dilute the 20× Wash solution with deionized water in the volume ratio of 1:19. (e.g. Add 10mL 20× Wash solution into 190mL deionized water.

## Assay Procedure

**Note:**

1. The temperature of all reagents and strips required was raised to room temperature (20-25°C) before use.
2. All reagents were returned (2-8°C) immediately after use.
3. The reproducibility in the ELISA assay is highly dependent on the consistency of the wash plate, and the correct plate wash operation is the point in the ELISA assay procedure.
4. During all incubations, avoid light exposure and seal the microplate with a cover film.

**Procedure:**

1. Take all reagents out at room temperature (20-25°C) for more than 30min. Shake gently before use.
2. Get the microwells needed out and return the rest into the zip-lock bag at 2-8°C immediately.
3. Number: Number every microwell position and all standards and samples should be run in duplicate. Record the standards and samples positions.
4. Add standard /sample: Add 50µl of standard solution or prepared sample to corresponding wells. Add 50ul of antibody solution to each well. Mix gently by shaking the plate manually and incubate for 30min at 25°C with cover.
5. Wash: Remove the cover gently and pour the liquid out of the wells and rinse the microwells with wash solution 5 times. Absorb the residual water with absorbent paper (the rest air bubble can be eliminated with unused tips).
6. Add Enzyme conjugate: Add 100ul of enzyme conjugate to each well. Mix gently by shaking the plate manually and incubate for 30min at 25°C with cover.
7. Discard the solution. Repeat the wash as in step 5.
8. Coloration: Add 100µl of substrate solution to each well and incubate for 15min at 25°C with cover (or in dark place).
9. Add 50 µl of Stop Solution to each well, gently shake and mix, set the microplate reader at 450nm

(recommended to use dual-wavelength 450/620nm detection), please read the data within 5min.

## Calculation

Percentage absorbance: The mean values of the absorbance values obtained from the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%.

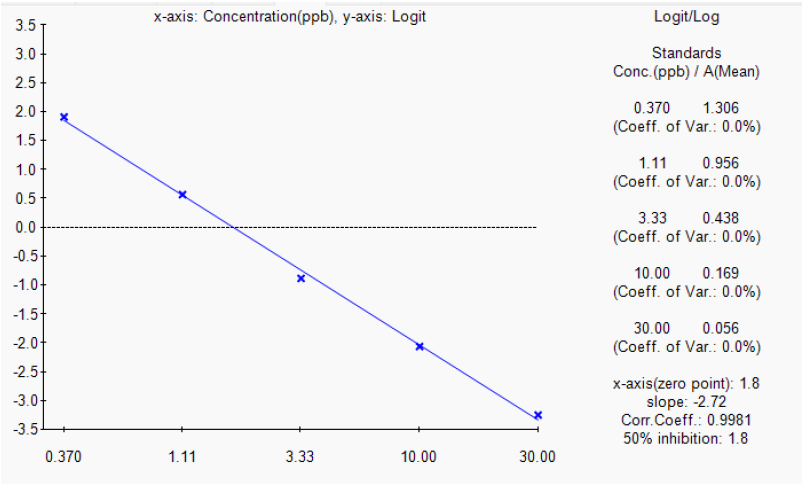
Absorbance (%) = (B/B0) *100%

B - absorbance of standards or samples

B0 - absorbance of zero standard (0ng/mL)

**Note:** The standard curve is drawn by taking the percent absorbance of the standard as the ordinate and the logarithm of the ferulic acid standard concentration (ppb) as the abscissa. The percent absorbance of the sample is substituted into the standard curve, and the concentration corresponding to the sample is read from the standard curve, and the corresponding dilution factor is multiplied by the actual concentration of xylazine in the sample. If professional analysis software is used for calculation, it is more convenient for accurate and rapid analysis of many samples.

## Typical Standard Curve




## Precision

Intra-plate coefficient of variation: <10%

Inter-plate variation coefficient: <10%

## Sensitivity

Kit sensitivity: < 0.37ng/mL