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User's Manual

Clonazepam ELISA Kit

REF **DEIANJ29**

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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

The Clonazepam ELISA Test Kit is a competitive enzyme immunoassay for the quantitative analysis of Clonazepam in human urine, whole blood and oral fluid.

General Description

Clonazepam is a medication used to prevent and treat seizures, panic disorder, and for the movement disorder known as akathisia. It is a tranquilizer of the benzodiazepine class.

Principles of Testing

The method is based on an indirect competitive ELISA assay. The drug of interest has been coated in the plate wells. During the analysis, sample is added along with the primary antibody specific for the target drug. If the target is present in the sample, it will compete for the antibody, thereby preventing the antibody from binding to the drug attached to the well. The secondary antibody, tagged with a peroxidase enzyme, targets the primary antibody that is complexed to the drug coated on the plate wells. The resulting color intensity, after addition of substrate, has an inverse relationship with the target concentration in the sample.

Reagents And Materials Provided

1. Microplate: 96 well polystyrene microplate (12 strips of 8 wells) coated with Clonazepam;
2. Clonazepam Standards
3. Antibody Solution
4. HRP Conjugate Antibody
5. Sample Diluent
6. Wash Solution
7. TMB Solution A
8. TMB Solution B
9. TMB Stop Solution
10. Microtiter plate sealers
11. Plastic Sealable Bag

Materials Required But Not Supplied

1. Validated microplate reader.
2. Homogenizer
3. Electronic balance
4. Centrifuger

5. Shaker for microtiter plates (optional)
6. Organomation
7. Vortex genie
8. Validated adjustable micropipettes, single and multichannel.
9. Timer

Storage

Store the kit at 2 - 8°C.

Specimen Collection And Preparation

Homogenized, centrifuged, evaporated, diluted for assay

Assay Procedure

1. Add 50µl of the standard solutions or samples (sample extracts) into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.
2. Add 50µl of enzyme conjugate solution to the individual wells successively using a multi-channel pipette or a stepping pipette.
3. Add 100µl of antibody solution to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for about 30 seconds. Be careful not to spill contents.
4. Incubate the strips for 40 minutes at room temperature.
5. After incubation, remove the covering and vigorously shake the contents of these wells into a sink. Wash the strips three times using the 1X washing buffer solution. Use at least a volume of 260 µl of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.
6. Dispense 50 µl of TMB Solution A and 50 µl TMB Solution B into each well. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for about 30 seconds. Incubate the strips for 15-20 minutes at room temperature. Protect the strips from direct sunlight.
7. Add 50 µl of stop solution to the wells in the same sequence as for the substrate solution.
8. Read the absorbance at 450 nm and 630 nm using a microplate ELISA photometer within 5 minutes after the addition of the stopping solution.