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Product Information

Tricyclics ELISA

Catalog Number **SE120131**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

The Tricyclic ELISA kit is a sensitive *in vitro* test to detect the presence of tricyclic antidepressants in forensic samples such as whole blood, serum, plasma, and urine. It avoids extraction of urine or blood samples for measurement. It employs a tricyclic antidepressant (TCA) directed antiserum. Due to the proprietary method of orienting the antibody on the polystyrene microplate much higher sensitivity is achieved compared to passive adsorption. This allows an extremely small sample size, reducing matrix effects and interference with binding protein(s) or other macromolecules. The technique is sensitive to 2 ng/mL.

The Tricyclics ELISA Kit is based upon the competitive binding to antibody of enzyme labeled antigen and unlabeled antigen, in proportion to their concentration in the reaction mixture. An aliquot of a diluted unknown specimen is incubated with a 100 µL dilution of enzyme (Horseradish peroxidase) labeled Tricyclics derivative in microplate wells, coated with fixed amounts of oriented high affinity purified polyclonal antibody. The wells are washed thoroughly and a chromogenic substrate added. The color produced is stopped using a dilute acid stop solution and the wells read at 450 nm. The intensity of the color developed is inversely proportional to the concentration of drug in the sample.

Components

Materials Provided	96 Tests
Coated Microwells	12 x 8 x 1
Tricyclics Conjugate	15 mL
Negative Standard	2 mL
Immunalysis Positive Standard	2 mL
TMB Substrate	30 mL
Stop Solution	25 mL

Reagents and Equipment Required but Not Provided.

- Distilled or deionized water
- Precision pipettes
- Disposable pipette tips
- ELISA reader capable of reading absorbance at 450 nm
- Absorbent paper or paper towel
- Graph paper

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Sample Preparation

1. The Tricyclics ELISA Kit is to be used with human samples such as whole blood, serum, urine, and plasma. All possible applications of this assay have not been tested. The cutoff criteria are important in deciding the sample dilution. It is recommended to dilute most blood samples either 1:5 or 1:10 depending on the cutoff used by the laboratory.
2. Specimens to which sodium azide has been added affect the assay.
3. Urine samples should be stored at 2–4 °C until use. Samples should be well mixed before assay. Repeated freezing and thawing should be avoided. Urine samples should be shipped refrigerated with ice packs or equivalent.

Storage/Stability

Store the kit at 2–8 °C. Keep microwells sealed in a dry bag with desiccants. The reagents are stable until expiration of the kit. Do not expose test reagents to heat, sun or strong light.

Procedure

Notes: The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

It is recommended that serum samples be run in duplicate.

Optimal results will be obtained by strict adherence to this protocol. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are essential. Any deviation from this may yield invalid data.

Bring all specimens and kit reagents to room temperature (18–26 °C) and gently mix.

1. Add 25 µL of calibrators and standards to each well in duplicate.
2. Add 25 µL of the diluted specimens to each well. It is recommended to run specimens in duplicate.
3. Add 100 µL of the Enzyme Conjugate to each well. Tap the sides of the plate holder to ensure proper mixing.
4. Incubate for 60 minutes at room temperature (20–25 °C) preferably in the dark, after addition of enzyme conjugate to the last well.
5. Wash the wells 6 times with 350 µL of distilled water using either a suitable plate washer or wash bottle taking care not to cross contaminate wells. If testing samples containing abnormally high amounts of hemoglobin (some postmortem samples), use 10 mM Phosphate Buffered Saline, pH 7.0–7.4. This will lower potential non-specific binding of hemoglobin to the well, thus lowering background color.
6. Invert wells and vigorously slap dry on absorbent paper to ensure all residual moisture is removed. This step is critical to ensure that residual enzyme conjugate does not skew results. If using an automated system, ensure that the final aspiration on the wash cycle aspirates from either side of the well
7. Add 100 µL of Substrate reagent to each well and tap sides of plate holder to ensure proper mixing.
8. Incubate for 30 minutes at room temperature, preferably in the dark.
9. Add 100 µL of Stop Solution to each well, to change the blue color to yellow.
10. Measure the absorbance at a dual wavelength of 450 nm and 650 nm. Compare average absorbance readings obtained from each unknown specimen with the average absorbance obtained from the Positive Reference Standard
11. Wells should be read within 1 hour of yellow color development

Results
If the average sample absorbance is equal to or less than the average absorbance of the laboratory positive reference standard the sample is POSITIVE for Tricyclics.

If the average sample absorbance is greater than the average absorbance of the laboratory positive reference standard the sample is called NEGATIVE for Tricyclics.

Alternatively a dose response curve can be established by plotting standard concentration (abscissa) against corresponding absorbance (ordinate). Values for unknown samples are obtained by interpolation from the curve. The following data represent a typical dose/response curve:

Nortriptyline (pg/well)	Absorbance
0	3.23
62.5	1.70
125	1.28
250	0.94

The dose/response curve shown should not be used in assay calculations. It is recommended that at least one in-house positive quality control sample be included with every assay run. A dose response curve or a cutoff calibrator should be run with every plate.

Product profile
Precision
The precision of the Tricyclic ELISA kit has been verified by assessment of the mean, standard deviation (SD) and coefficients of variation (CV) in data resulting from repetitive assays.

Intra-assay Precision
Intra-assay precision was determined with reference controls. A 0, 62.5, 125, and 250 pg/well spikes of Nortriptyline standard (0, 25, 50, and 100 ng/mL diluted 1:10 and 25 µL of diluted sample used) was assayed eight times in the same assay. The results are tabulated in Table 1.

Nortriptyline (pg/well)	Mean Abs	S.D.	C.V %
0	3.227	0.048	1.49
62.5	1.697	0.100	5.91
125	1.279	0.072	5.64
250	0.937	0.045	4.78

Sensitivity
Assay sensitivity based on the minimum Nortriptyline concentration required to produce a four standard deviation from assay Ao is 12.5 pg of Nortriptyline per well.

Specificity
The specificity and cross-reactivities of the Tricyclic ELISA kit was determined for each of the compounds listed.

Cross-Reactivities Compound	Approx. ng/mL equivalent to ng of Nortripyline	Cross-Reactivities
Nortriptyline	25	100
Amitriptyline	12.5	200
Desipramine	12.5	200
Imipramine	12.5	200
Trimipramine	50	50
Clomipramine	60	40
Norclomipramine	125	20
Doxepin	160	15
Nordoxepin	160	15
Protriptyline	100	25
Cyclobenzaprine	30	83
Chlorpromazine	60	40
Diphenhyramine	10,000	0.25
Quetiapine	10,000	0.25

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