DATA SHEET

IDEL-F083
Enzyme Immunoassay for the Detection of Olanzapine in Urine or Serum_

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PRODUCT DESCRIPTION

The IDELISA™ Forensic Olanzapine ELISA Kit is a competitive enzyme immunoassay for the detection of anti-psychotic,
Olanzapine in urine or blood. Equine and human administrations for Olanzapine are positive for up to 24 hours using this screening
test.
The IDELISA™ Forensic Olanzapine ELISA Kit was designed for screening purposes and is intended for forensic or research use
only (NOT FOR THERAPEUTIC USE). All suspect samples should be confirmed by a quantitative method such as Gas
Chromatography-Mass Spectrometry (GC-MS).
This kit is a simple, rapid, sensitive and cost-effective screening method. The unique features of the kit are:

_ Consistent, reproducible results

_ High sensitivity 50% B/Bo (0.50 ng/ml)

_ Assay Range 0.25 ng/ml – 100.0 ng/ml

_ Fast, assay times can be less than 2 hours

Like most ELISA assays, this kit relies on a Horseradish Peroxidase (HRP) conjugated antibody and the TMB (3,3´,5,5´-
tetramethylbenzidine) substrate. TMB is a chromogen that yields a blue color when oxidized with hydrogen peroxide (catalyzed by
HRP) that has major absorbances at 370 nm and 652 nm. The color then changes to yellow with the addition of acid with maximum
absorbance at 450 nm. The relative amount of Olanzapine in the sample is directly proportional to the amount of signal that is
obtained at 450 nm.
This kit contains materials for the extraction and quantitative detection of Olanzapine in urine or serum.

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PROCEDURE OVERVIEW

The method is based on a competitive colorimetric ELISA assay. The drug of interest has been coated in the plate wells. The sample
of interest is added along with a primary antibody specific for the target drug. If the target is present in the sample, it will compete for
the primary antibody, thereby preventing the antibody from binding to the drug attached to the well. After incubation, the sample is
removed, the wells are washed and a second antibody, which is directly conjugated to HRP, is added. Signal is generated by reaction
with the TMB substrate as described above. The intensity of the signal (measured at 450 nm) is directly proportional to the amount of
Olanzapine in the sample. Dilutions of the Olanzapine Spike in Standard are used to construct a standard curve, from which the
concentration of Olanzapine in the samples are determined by extrapolation. This is described in more detail in Section, “Olanzapine
Concentration Calculations.”

KIT REAGENTS SUPPLIED

The kit has the capacity for 96 determinations (96 wells) or testing of 42 samples in duplicate (assuming 12 wells for standards).
Return any unused microwells to the foil bag and reseal them with the desiccant provided in the original package. Store the kit at 2-

8°C*. The shelf life is noted on the kit label, when the kit and components are properly stored.

Kit Contents	Amount	Storage
Olanzapine-Coated Microtiter Plate	1 x 96-well plate (8 wells x 12 strips)	2-8°C
Olanzapine Antibody #1	15 ml	2-8°C *
1000 ng/ml Standard	1.0 ml	2-8°C*
100X HRP-Conjugated Antibody #2	300 µl	2-8°C *
Antibody #2 Diluent **	20 ml	2-8°C
20X Wash Solution **	28 ml	2-8°C
Stop Buffer **	20 ml	2-8°C
TMB Substrate **	12 ml	2-8°C
10X PBS **	25 ml	2-8°C

* If you are not planning to use the kit for over 3 months, store Olanzapine Antibody #1, Standard, and 100X HRPConjugated
Antibody #2 at -20°C.
** These components are interchangeable between IDELISA™ Forensic Kits as long as they are used before the expiration dates on
the individual vials.

MATERIALS REQUIRED BUT NOT PROVIDED

- Microtiter plate reader (450 nm)
- 10, 20, 100 and 1000 µl pipettes
- Multi-channel pipette: 50-300 µl (Optional)
- Distilled deionized water
- Vortexer
- Incubator

SENSITIVITY (Detection Limit)

Sample Type	Detection Limit (ng/ml or ppb)
Urine	2.5
Serum	2.5

SPECIFICITY (Cross Reactivity)

Drug	Sensitivity	Cross reactivity
Olanzapine	0.50 ng/ml	100%
Clozapine	0.50 ng/ml	100%
Imipramine	10,000 ng/ml	0.01%
Acepromazine	10,000 ng/ml	0.01%
Desipramine	10,000 ng/ml	0.01%
Nortriptyline	10,000 ng/ml	0.01%
Sertraline	10,000 ng/ml	0.01%
Thioridazine	10,000 ng/ml	0.01%
Mesoridazine	10,000 ng/ml	0.01%
Trifluomeperazine	10,000 ng/ml	0.01%
Prochlorperazine	10,000 ng/ml	0.01%
Butaperazine	10,000 ng/ml	0.01%
Ipsapirone	> 10,000 ng/ml	< 0.01%
Geprione	> 10,000 ng/ml	< 0.01%
Trazodone	> 10,000 ng/ml	< 0.01%
Nefazodone	> 10,000 ng/ml	< 0.01%
Risperidone	> 10,000 ng/ml	< 0.01%
OH- Risperidone	> 10,000 ng/ml	< 0.01%
Buspirone	> 10,000 ng/ml	< 0.01%

For *in vitro research use*. CAUTION: Not for human or animal therapeutic use.
Uses other than the labeled intended use may be a violation of local law.

Fluoxetine	> 10,000 ng/ml	< 0.01%
Paroxetine	> 10,000 ng/ml	< 0.01%
Thiethlyperazine	> 10,000 ng/ml	< 0.01%

WARNINGS AND PRECAUTIONS

ID Labs™ strongly recommends that you read the following warnings and precautions to ensure your full awareness of ELISA techniques and other details you should pay close attention to when running the assays. More information can also be found in the Troubleshooting section. **Periodically, optimizations and revisions are made to the kit and manual. Therefore, it is important to follow the version of the protocol included with the kit.**

Additional Technical Hints

- Do not use the kit past the expiration date.
- Do not intermix reagents from different kits or different lots. **Antibodies and plates are kit and lot specific.** Make sure that the standards, detection antibody, avidin-HRP, and diluent are mixed in correct volumes.
- Make sure that the 100X HRP-Conjugated Antibody #2 and Antibody #2 Diluent are mixed in correct volumes.
- Try to maintain a laboratory temperature of (20 – 25°C / 68 – 77°F). Avoid running assays under or near air vents, as this may cause excessive cooling, heating and/or evaporation. Also, do not run assays in direct sunlight, as this may cause excessive heat and evaporation. Cold bench tops should be avoided by placing several layers of paper towel or some other insulation material under the

assay plates during incubation.
• Make sure you are using only distilled deionized water since water quality is very important.
• When pipetting samples or reagents into empty microtiter plate, place the pipette tips in the lower corner of the well, making
contact with the plastic.
• Incubations of assay plates should be timed as precisely as possible. Be consistent when adding standards to the assay plate. Add
your standards first and then your samples.
• Add standards to plate only in the order from low concentration to high concentration as this will minimize the risk of
compromising the standard curve.
• Always refrigerate plates in sealed bags with a desiccant to maintain stability. Prevent condensation from forming on plates by
allowing them to equilibrate to room temperature (20 – 25°C / 68 – 77°F) before opening (plates provided in package containing
desiccant).

NOTE: ID Labs™ makes no warranty of any kind, either expressed or implied, except that the materials from which such products
are made are of standard quality. There is no warranty of merchantability of this product, or of the fitness of the product for any
purpose. ID Labs™ shall not be liable for any damages, including special or consequential damage, or expense arising directly or
indirectly from the use of this product.

SAMPLE PREPARATION
Be sure samples are properly stored. In general, samples should be refrigerated at 2-4°C for no more than 1-2 days. Freeze samples to
a minimum of -20°C if they need to be stored for a longer period. Frozen samples can be thawed at room temperature (20 – 25°C / 68
– 77°F) or in a refrigerator before use. Preparation protocols for other samples can be made available upon request.
Preparation of 1X PBS: Mix 1 volume of the 10X PBS with 9 volumes of distilled water.
Urine
1. Centrifuge 0.5 ml of the urine sample at 4,000x g for 5 minutes.
2. Take out 200 µl of the supernatant and add 800 µl of 1X PBS and mix well.
3. Use 100 µl of the diluted supernatant per well in the assay.
Note: Dilution factor: 5
If the Olanzapine concentration in the sample is too high, the sample can be further diluted in 1X PBS, and the assay re-run.

Serum
1. Blood should be collected without anticoagulant and left at room temperature for 3 hours or at 4°C overnight, to clot.
2. Centrifuge at 3,000 g for 10 minutes at 4°C.
3. Take 200 µl of the serum from the upper layer of the blood sample. Add 800 µl of 1X PBS and mix well.
4. Use 100 µl of the diluted serum per well in the assay.
Note: Dilution factor: 5
If the Olanzapine concentration in the sample is too high, the sample can be further diluted in 1X PBS and the assay re-run.
PREPARATION OF REAGENTS

IMPORTANT: All reagents should be brought up to room temperature before use (1 – 2 hours at 20 – 25°C / 68 – 77°F); Make sure
you read “Warnings and Precautions” section. Solutions should be prepared just prior to ELISA test. All reagents should be mixed by

gently inverting or swirling prior to use. Prepare volumes that are needed for the number of wells being run. Do not return the reagents
to the original stock tubes/bottles. Using disposable reservoirs when handling reagents can minimize the risk of contamination and is
recommended.

1. **Preparation of 1X HRP-Conjugated Antibody #2**
Mix 1 volume of 100X HRP-Conjugated Antibody #2 with 99 volumes of Antibody #2 Diluent.

2. **Prepare 1X PBS**
Mix 1 volume of the 10X PBS with 9 volumes of distilled water.
3. **Preparation of Standards**
Dilute 1000 ng / ml Standard within 1-100 ng /ml range using 1X PBS before adding to the wells. Use 1X PBS alone as a negative
control standard.
4. **Preparation of 1X Wash Solution**
Mix 1 volume of the 20X Wash Solution with 19 volumes of distilled water.
ASSAY PROCEDURE
Label the individual strips that will be used and aliquot reagents as in the following example:

Component	Volume per Reaction	24 Reactions
Olanzapine Antibody #1	100 µl	2.4 ml
1X HRP-Conjugated Antibody #2	150 µl	3.6 ml
1X Wash Solution	2.0 ml	48 ml
Stop Buffer	100 µl	2.4 ml
TMB Substrate	100 µl	2.4 ml

1. Add 100 µl of each Olanzapine standards in duplicate into different wells **(Add standards to plate only in the order from low
concentration to high concentration).**
2. Add 100 µl of each sample in duplicate into different sample wells.
3. Add 100 µl of Antibody #1 and mix well by gently rocking the plate manually for 1 minute.
4. Incubate the plate for 30 minutes at room temperature (20 – 25°C / 68 – 77°F).
5. Wash the plate 3 times with 250 µl of 1X Wash Solution. After the last wash, invert the plate and gently tap the plate dry on paper
towels **(Perform the next step immediately after plate washings. Do not allow the plate to air dry between working steps).**
6. Add 150 µl of 1X HRP-Conjugated Antibody #2. Incubate the plate for 30 minutes at room temperature (20 – 25°C / 68 – 77°F)
(Avoid direct sunlight and cold bench tops during the incubation. Covering the microtiter plate while incubating is recommended).
7. Wash the plate 3 times with 250 of µl 1X Wash Solution. After the last wash, invert the plate and gently tap the plate dry on paper
towels **(Perform the next step immediately after plate washings. Do not allow the plate to air dry between working steps).**
8. Add 100 µl of TMB substrate. Time the reaction immediately after adding the substrate. Mix the solution by gently rocking the
plate manually for 1 minute while incubating. **(Do not put any substrate back to the original container to avoid any potential
contamination. Any substrate solution exhibiting coloration is indicative of deterioration and should be discarded. Covering
the microtiter plate while incubating is recommended).**
9. After incubating for 10-30 minutes at room temperature (20 – 25°C / 68 – 77°F), add 100 µl of Stop Buffer to stop the enzyme
reaction.

10. Read the plate as soon as possible following the addition of Stop Buffer on a plate reader with 450 nm wavelength **(Before reading, use a lint-free wipe on the bottom of the plate to ensure no moisture or fingerprints interfere with the readings).**

CALCULATION OF RESULTS – OLANZAPINE CONCENTRATION CALCULATION

A standard curve can be constructed by plotting the mean relative absorbance (%) obtained from each reference standard against its concentration in ng/ml on a logarithmic curve.

$$\text{Relative absorbance (\%)} = \frac{\text{absorbance standard (or sample) x 100}}{\text{absorbance zero standard}}$$

absorbance standard (or sample) x 100
absorbance zero standard

Use the mean relative absorbance values for each sample to determine the corresponding concentration of the tested drug in ng/ml from the standard curve.

We would recommend a standard curve that is made up of at least 6 dilutions of standards, including zero (0), in duplicate.