



## IDELISA™ Forensic Cyproheptadine ELISA Kit

IDEL-F029

Enzyme Immunoassay for the Detection of Cyproheptadine in Urine or Blood

### PRODUCT DESCRIPTION

The IDELISA™ Forensic Cyproheptadine ELISA Kit is a competitive enzyme immunoassay for the detection of Cyproheptadine in urine or blood. Cyproheptadine is used to treat allergic reactions, specifically hay fever.

The IDELISA™ Forensic Cyproheptadine 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 an analytical method such as Gas Chromatography-Mass Spectrometry (GC-MS). For In Vitro Research Use Only.

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 (< 1.0 ng/ml)
- Assay Range 0.05 ng/ml – 10 ng/ml
- 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 Cyproheptadine in the sample is directly proportional to the amount of signal that is obtained at 450 nm.

### 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 Cyproheptadine in the sample. Dilutions of the Cyproheptadine Standard, are used to construct a standard curve, from which the concentration of Cyproheptadine in the samples are determined by extrapolation. This is described in more detail in Section "Cyproheptadine 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 is properly stored.

Kit Contents	Amount	Storage
Cyproheptadine Coated Microtiter Plate	1 x 96-well plate (8 wells x 12 strips)	2-8°C
Cyproheptadine Antibody #1	15 ml	2-8°C *
1000 ng/ml Cyproheptadine 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 Cyproheptadine Antibody #1 & 100X HRP-Conjugated 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.

**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.

# Instructions For Use Data Sheet IDEL-F029

Rev Date:  
Mar 29 2012

Revision: 2

Page 2 of 6

## MATERIALS REQUIRED BUT NOT PROVIDED

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

## SENSITIVITY (Detection Limit)

Sample Type	Detection Limit (ng/ml or ppb)
Urine	0.25
Serum	0.25

## SPECIFICITY (Cross Reactivity)

Drug	Sensitivity	Cross reactivity
Cyproheptadine	<1.0 ng/ml	100%

## 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 packaging contain desiccant).

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## 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 temps (20 – 25°C / 68 – 77°F) or in a refrigerator before use. Preparation protocols for other samples can be made available upon request.

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# Instructions For Use Data Sheet IDEL-F029

Rev Date:  
Mar 29 2012

Revision: 2

Page 3 of 6

**Preparation of 1X PBS:** Mix 1 volume of the 10X PBS with 9 volumes of distilled water.

## 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 3,000x 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, mix well.
4. Use 100 µl of the sample per well in the assay.

**Note:** Dilution factor: 5

If the Cyrproheptadine concentration in the sample is too high the sample can be further diluted with 1X PBS.

## 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, mix well.
3. Use 100 µl of the sample per well in the assay.

**Note:** Dilution factor: 5

If the Cyrproheptadine concentration in the sample is too high the sample can be further diluted with 1X PBS.

## 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. Preparation of 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 0.05-10 ng/ml range using 1X PBS prior to adding to wells. Use 1X PBS as 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
Cyrproheptadine 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 Cyrproheptadine 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).

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<b>Instructions For Use Data Sheet IDEL-F029</b>		
Rev Date: Mar 29 2012	Revision: 2	Page 4 of 6

5. Wash the plate 3 times with 250  $\mu$ l 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  $\mu$ l 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  $\mu$ l 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  $\mu$ l 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  $\mu$ l 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 – CYPROHEPTADINE 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.

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

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 8 samples in duplicate.**

# Instructions For Use Data Sheet IDEL-F029

Rev Date:  
Mar 29 2012

Revision: 2

Page 5 of 6

## TROUBLESHOOTING

### No Color Development or No Signals with Standards

Possible Causes	Recommended Action
Reagents were used in the wrong order or a step was skipped.	Follow the protocol carefully and repeat the assay.
Wrong antibodies were used. Either Detection Antibody or Avidin-HRP was prepared incorrectly or has deteriorated.	Make sure that the antibodies used are the ones that came with the kit. All antibodies are kit- and lot-specific. Make sure that the Detection Antibody, Avidin-HRP and diluent are mixed in correct volumes.
TMB Substrate has deteriorated.	Use a new set of TMB substrate. Note, if TMB substrate shows any color before use, it should not be used for the assay.

### Low Optical Density (OD) Readings

Possible Causes	Recommended Action
Reagents were expired or mixed with a different lot number.	Verify the expiration dates and lot numbers.
Wash solution was prepared incorrectly.	Verify that the solution was prepared as described in the protocol.
Too many wash cycles were used.	Make sure to use the number of washes per the protocol instruction.
Incubation times were too short.	Follow protocol and ensure accurate incubation time.
Lab temperature was too low.	Maintain the lab room temperature within 20°–25°C. Do not run assays under air conditioning vents or near cold windows.
Reagents and plates were too cold.	Make sure that the plates and reagents are brought up to room temperature.
Reader was at wrong wavelength, or reader was malfunctioning.	Make sure that the wavelength is set to 450 nm and read the plate again. Verify reader calibration and lamp alignment.
Excessive kit stress has occurred.	Check records to see how many times the kit has cycled from the refrigerator. Check to see if the kit was left at extreme temperatures for too long.
Assay plates were compromised.	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 while in the packaging.

### High Background or High Optical Density (OD) Readings

Possible Causes	Recommended Action
Poor quality water was used in wash solution.	If water quality is questionable, try substituting an alternate source of distilled deionized water to prepare the wash solution.
Substrate solution has deteriorated.	Make sure that the substrate is colorless prior to addition to the plate.
There was insufficient washing or poor liquid handling technique.	Use the number of washes per the protocol instruction. Make sure that 250 µL of wash solution is dispensed per well per wash. If you use a multichannel pipette or robotic liquid handling system, verify its performance; have the system repaired if any ports drip, dispense or aspirate poorly.
Reader was malfunctioning or not blanked properly. This is a high possibility if the OD readings were high and the color was light.	Verify the reader's performance using a calibration plate and check the lamp alignment. Verify the blanking procedure, if applicable, and re-blank.
Lab temperature was too high.	Maintain the room temperature within 20°–25°C. Avoid running assays near heat sources or in direct sunlight.
Reagents were intermixed, contaminated or prepared incorrectly.	Ensure that the correct reagents were used, that working solutions were prepared correctly and that contamination has not occurred.

# Instructions For Use Data Sheet IDEL-F029

Rev Date:  
Mar 29 2012

Revision: 2

Page 6 of 6

## High Intra-Plate or Inter-Plate Variance

Possible Causes	Recommended Action
Inconsistent time was taken when adding standards, reagents or samples within and/or between plates.	Make sure that all materials are set up and ready to use. Use a multichannel pipette to add reagents to multiple wells whenever possible. Do not interrupt procedure while adding standards, reagents and samples.
Multichannel pipette was not functioning properly.	Verify pipette calibration and check that tips are on tight. Be sure all channels of the pipette draw and dispense equal volumes.
There was inconsistent washing or poor liquid handling technique.	Use the number of washes per the protocol instruction. Make sure that 250 $\mu$ l of wash solution is dispensed per well per wash. If you use a multichannel pipette or robotic liquid handling system, verify its performance; have the system repaired if any ports drip, dispense or aspirate poorly.
Inconsistent incubation times occurred from plate to plate.	Time each plate separately to ensure consistent incubation times.
Pipette was inaccurate.	Check the pipette calibration. Verify that pipette tips are on tight before use and that all channels draw and dispense equal volumes.
Kit plates, reagents, standards and samples were at different temperatures.	Make sure to allow sufficient time for kit plates, reagents, standards and samples to come to room temperature. Larger volumes will require longer equilibration time. If using a water bath to hasten equilibration, make sure that it is maintained at room temperature; do not use a warm water bath to warm reagents, samples and kit standards.
Reagents used were intermixed from different kit lots, or the kits were of different expiration dates.	Carefully label each user-prepared reagent to make sure that the reagents are not intermixed. Kits with different expiration dates might generate different range of OD readings, however, the relative absorbance values will typically be comparable. In general, a value of less than 1.0 reading for the highest standard may indicate deterioration of reagents.

## One or More of the Standard Curve Points Are Out of Range

Possible Causes	Recommended Action
Standards were added in wrong order or recorded in wrong position.	Make sure that the standards are applied and recorded correctly.
Standards were contaminated or intermixed with other standards.	Prepare a new set of standards. Always add standards to plate in the order from low concentration to high concentration.
There was inconsistent washing or poor liquid handling technique.	Use the number of washes per the protocol instruction. Make sure that 250 $\mu$ l of wash solution is dispensed per well per wash. If you use a multichannel pipette or robotic liquid handling system, verify its performance; have the system repaired if any ports drip, dispense or aspirate poorly.
Inconsistent time was taken to add standards and reagents to plate.	Make sure all materials are set up and ready to use. Add standards to plate only in the order from low concentration to high concentration at undisturbed constant pace. Use a multichannel pipette to add reagents to multiple wells simultaneously to increase consistency.
Multichannel pipette was not functioning properly.	Verify pipette calibration and check that tips are on tight. Be sure all channels of the pipette draw and dispense equal volumes.

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