

ELISA kits available from ADI (see details at the web site)

DE-100010	Clenbuterol ELISA kit, 96 tests (For Urine, Serum, Feed, Meat, Liver)
DE-100020	Ractopamine ELISA kit, (For Liver, Urine, Feed), 96 tests
DE-100030	Salbutamal ELISA kit, For Urine, Tissue, Feed, Animal Tissue, Aquatic, Honey, Intestine,, 96 tests
DE-100040	Chloramphenicol ELISA kit, 96 tests (For Animal Tissue, Aquatic, Honey, Intestine, Urine, Egg, Milk, Serum)
DE-100050	Florfenicol ELISA kit (For Animal Tissue, Aquatic, Honey), 96 tests
DE-100060	Nitrofurantol (AMOZ) ELISA kit (For Fish, Shrimp, Honey, Chicken/Liver), 96 tests
DE-100070	Nitrofurantol (AHD) ELISA kit, (For Fish, Shrimp, Honey, Chicken/Liver), 96 tests
DE-100075	Nitrofurantol (SEM) ELISA kit (Honey, Fish, Shrimp, Chicken/Liver, Fish/Shrimp), 96 tests
DE-100080	Nitrofurantol (AOZ) ELISA kit (For Fish, Shrimp, Honey, Chicken/Liver), 96 tests
DE-100090	Sulfonamides Residues (SAs) ELISA kit, (For Chicken/Liver, Pork/Liver, Honey/Egg, Serum/Urine, Milk), 96 tests
DE-100100	Sulfamethazine (SM2) ELISA kit, 96 tests (For Chicken/Liver, Pork/Liver, Honey/Egg, Serum/Urine, Milk)
DE-100110	Sulfamethoxydiazine (SMD) ELISA kit, (For Chicken/Liver, Pork/Liver, Honey/Egg, Serum/Urine), 96 tests
DE-100120	Quinolones (QNS) ELISA kit (For Pork/Liver, Chicken/Liver, Shrimp, Fish, Serum, Honey), 96 tests
DE-100130	Enrofloxacin ELISA kit (For Pork/Liver, Chicken/Liver, Shrimp, Fish, Serum, Honey), 96 tests
DE-100140	Ampicillin ELISA kit, (For Pork/Liver, Chicken, Duck, Shrimp, Fish, Honey, Milk), 96 tests
DE-100150	Benzyl Penicillin ELISA kit, (For Pork/Liver, Chicken, Duck, Shrimp, Fish, Honey, Milk), 96 tests
DE-100160	Tylosin ELISA kit (For Meat, Liver, Honey, Egg), 96 tests
DE-100170	Trenbolone ELISA kit (For Animal Tissue, Aquatic, Urine), 96 tests
DE-100180	Diazepam ELISA kit (For Tissue, Urine, Feed), 96 tests
DE-100190	Diethylstilbestrol (DES) ELISA kit (Fish, Shrimp, Liver, Meat, Feed, Urine), 96 tests
DE-100200	Gentamicin ELISA kit (Chicken/Liver), 96 tests
DE-100130	Enrofloxacin ELISA kit, 96 tests (Chicken/Liver, Honey, Milk)
DE-100230	Olaquinox ELISA kit (Tissue) 96 tests
DE-100240	Sulfaquin-oxaline ELISA kit, (For Pork/Liver, Honey/Egg, Serum/Urine, Milk), 96 tests

Instruction Manual No. DE-100620

Isoxsuprine ELISA kit

ELISA KIT Cat. # DE-100620, 96 Tests

For Detecting Isoxsuprine in urine, blood, and oral fluid

For In Vitro Research Use Only (RUO)

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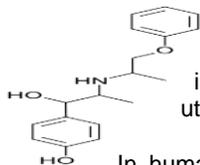
Isoxsuprine ELISA KIT Cat. DE-100620 # (96 tests)

Kit Components (96 tests)	Cat #
Isoxsuprine antibody coated strip plate, # (8x12 strip or 96 wells)	1 plate
Isoxsuprine Negative Control: (-ve control) 0.75mL #	1 vial
Isoxsuprine Positive Control: (+ve control) 0.75mL #	1 vial
Isoxsuprine-HRP Conjugate, 180X (200ul)	1 bottle
ELISA buffer, 40 ml #	1 bottle
Wash buffer (10X) 20 ml #	1 bottle
TMB Substrate Solution, 20 ml #950110-TM	1 bottle
Stop Solution, 20 ml	1 bottle
Plastic Resealable bag for the un-used antigen strips	1
Complete Instruction Manual # M-	1

Intended Use

ADI's Isoxsuprine ELISA test kit is used for the determination of trace quantities of Isoxsuprine and/or other metabolites in human urine, blood, and oral fluids. Isoxsuprine ELISA test kit is a qualitative one-step kit designed for use as a screening device for the detection of drugs and/or their metabolites. The kit was designed for screening purposes and is intended for forensic use only. It is recommended that all suspect samples be confirmed by a quantitative method such as gas chromatography/mass spectrometry (GC/MS). (This kit is for research use only and not for diagnostic, preventive or cure of the disease, RUO).

Introduction



Isoxsuprine (isoxsuprine hydrochloride) is a drug used as a vasodilator in humans (Duvadilan) and equines. Isoxsuprine is a beta-adrenergic agonist that causes direct relaxation of uterine and vascular smooth muscle via beta-2 receptors.

In humans, the drug is used to treat premature labor, for cerebral vascular insufficiency. The might increase heart rate, change in BP, and can cause irritation in GI tract.

Isoxsuprine is most commonly used to treat hoof-related problems in the horse, most commonly for laminitis and navicular disease, as its effects as a vasodilator are thought to increase circulation within the hoof to help counteract the problems associated with these conditions.

PERFORMANCE CHARACTERISTICS

Sensitivity

Compound	I-50 in EIA buffer in ng/mL
Isoxsuprine	0.47
Nylindrin	1.71

The term I-50 is used to define the sensitivity of the test. This number is derived from a standard curve generated with the drug in EIA Buffer. The drug concentration that shows 50% less color activity than the zero standard is considered to be the I-50.

Specificity

Compound	Compound concentration (ng/mL)	Isoxsuprine Equivalents (ng/mL)	% cross reactivity
Isoxsuprine	0.47	0.47	100
Nylindrin	1.67	0.47	28

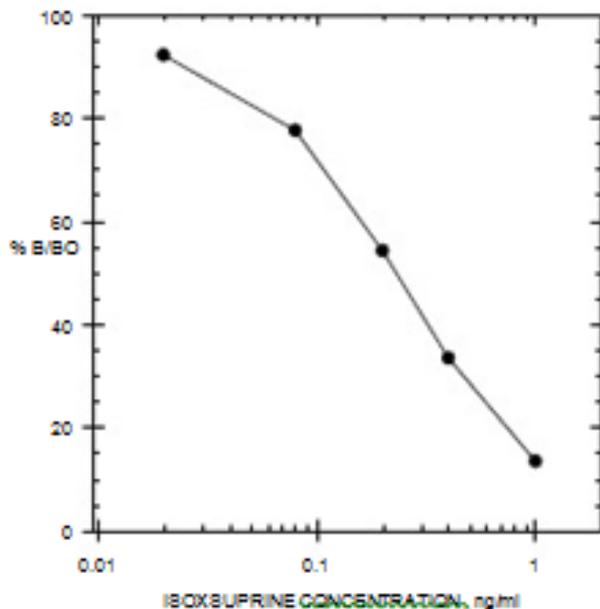
Note: Isoxsuprine equivalents represent 50% B/B0 assay displacement in EIA Buffer.

The compounds having cross-reactivity below 0.01% did not show any significant reaction up to 10µg/mL.

Cross Reactivity <0.01%.

ε-amino-n-caproic Acid; Amphetamine; Ascorbic Acid; Benzphetamine; 1-Benzylpiperazine; Clenbuterol; Diclofenac; Diethylpropion; Dimethyl Sulfoxide; Dipyrone; Ephedrine; Epinephrine; Ethyl p-amino-benzoate; Fenoterol; Flunixin; Furosemide; Glycopyrrolate; Hordenine; Hydrocortisone; Ibuprofen; Isoetharine; Isoproterenol; Mazindol; Metaproterenol; Metaraminol; Methamphetamine; Methocarbamol; Methoxyphenamine; Methylene Blue; 6α-Methylprednisolone; Naproxen; Orphenadrine; Oxyphenbutazone; Papaverine; Pentifylline; Pentoxifylline; Phenothiazine; Phenylbutazone; Polyethylene Glycol; Prednisolone; Procaine; Pyrantel; Ritodrine; Salbutamol; Salicylamide; Salicylic Acid; Terbutaline; Theobromine; Theophylline; Thiamine.

NOTE: The kit was designed for screening purposes only. It is recommended that all suspect samples be confirmed by a quantitative method such as GC/MS or HPLC.



Positive samples: Samples with an absorbance less than or equal to the laboratory's designated cutoff calibrator should be considered positive. All positive samples should be confirmed by a quantitative method such as GC/MS.

Negative samples: Samples with an absorbance greater than the laboratory's designated cutoff calibrator should be considered negative.

CALCULATION OF RESULTS:

Each laboratory should determine the cutoff level for their individual application. When possible, cutoff calibrators and/or standards should be prepared in the same matrix being tested. The positive and negative controls provided in the kit are for QC purposes only. The sole purpose of these controls is to verify that the test kit is performing properly. The controls are not intended for use as cutoff calibrators. The positive control is spiked at a high concentration and its approximate level can be found on the label.

MATERIALS AND EQUIPMENT REQUIRED

Deionized water, Precision pipettes that range from 10 μ L - 1000 μ L and disposable tips, Graduated cylinder, Plate cover or plastic film, Clean glassware, Microplate reader, shaker, 1 N HCl if red stop solution is not used. Note: It is not necessary to stop the reaction if reading immediately. Unstopped reactions should be read with a 650 nm filter. Cut-off calibrator.

Qualitative QC Positive Control: 5 X 750 μ L (synthetic human urine). 1-5 vials available upon request. Do not dilute.

Qualitative QC Negative Control: 5 X 750 μ L (synthetic human urine). 1-5 vials available upon request. Do not dilute.

Red Stop Solution: 100 mL (ready-to-use). Non acidic reagent used to stop the enzyme reaction. Available upon request. Product No. 301473.

PRINCIPLE OF THE TEST

Alpha Diagnostic's kit operates on the basis of competition between the drug or its metabolite in the sample and the drug-enzyme conjugate for a limited number of antibody binding sites. First, the sample or control is added to the microplate. Next, the diluted drug-enzyme conjugate is added and the mixture is incubated at room temperature. During this incubation, the drug in the sample or the drug-enzyme conjugate binds to antibody immobilized in the microplate wells. After incubation, the plate is washed 3 times to remove any unbound sample or drug-enzyme conjugate. The presence of bound drug-enzyme conjugate is recognized by the addition of Substrate (TMB). After 30 minute substrate incubation, the reaction is halted with the addition of Red Stop Solution. The test can be read visually or with a microplate reader equipped with a 650 nm filter. The extent of color development is inversely proportional to the amount of drug in the sample or control. In other words, the absence of the drug in the sample will result in a dark blue/purple color, whereas the presence of the drug will result in light pink to no color development.

PRECAUTIONS

- DO NOT use kits or components beyond expiration date.
- DO NOT mix conjugates and plates from different kit lots.
- All specimens should be considered potentially infectious. Exercise proper handling precautions.
- Keep plate covered except when adding reagents, washing or reading.
- Keep the controls frozen if storing longer than 10 days. Avoid repeated freeze-thaw cycles. Note: Some kits require controls to be stored frozen immediately upon receipt. Reference kit label for details.
- DO NOT smoke, eat or drink in areas where specimens or reagents are being handled.
- Do not use Sodium Azide with samples, standards and/or calibrators. Do not reuse wells; they are for one use only.

SAMPLE TREATMENT

Recommended minimum sample dilutions are listed below. These dilutions may change based on your laboratory's determination. All sample dilutions should be made in EIA Buffer.

a. **Urine:** A dilution with EIA Buffer may be necessary to reduce natural background as well as bring desired cutoff concentration within the assay range. b. **Whole blood:** A dilution of 1:5 (i.e. 1 part sample to 4 parts provided EIA Buffer) is recommended. c. Other Forensic sample types: Please contact ADI for information.

REAGENTS PREPARATION

1. **Dilute Wash buffer** 1:10 with water, (**20 ml stock in 180 ml distilled water**) Store diluted buffer at 4°C for 7 days.
2. **Drug-enzyme conjugate** 1:180 with EIA buffer (Dilute 180X before use). Prepare 2 ml per strip or about 20 ml for a full plate (100 ul stock conjugate and 1790 ul or 1.79 ml of ELISA buffer). Do not store diluted conjugate beyond the assay date.

All reagents must be at room temperature prior to their use.

STORAGE AND STABILITY

The microtiter well plate and all other reagents are stable at 2-8°C until the expiration date printed on the label. The whole kit stability is usually 6 months from the date of shipping under appropriate storage conditions. The unused portions of the standards should be stored at 2-8°C or stored frozen in small aliquots and should be stable for 3 months.

TEST PROCEDURE (ALLOW ALL REAGENTS TO REACH ROOM TEMPERATURE BEFORE USE).

Important: If you have not used this kit before, we recommend to use 1 or 2 strips to run the standards alone to get familiar with the test and not run the risk of making mistakes and lose sample or the whole kit.

Remove required number of coated strips and arrange them on the plate. Store unused strips in the bag. It is recommended to prepare a parallel replica plates containing all sample for quick transfer to the coated plate.

Label or mark the microtiter well strips to be used on the plate. Dilute the wash buffer with water (1:10). Dilute the stock conjugate (180 fold) in ELISA buffer

ASSAY PROCEDURE

1. Dispense 20 ul diluent in 1 well to be used as blank. Pipet **20 ul of calibrators, controls, and samples** into appropriate wells in *duplicate*.
2. Dispense **180 ul of DILUTED drug-enzyme conjugate** to each well. Cover the plate, mix gently for 5-seconds using **microplate shaker** and **incubate at room temp for 45 min**.
3. Aspirate the well contents and blot the plate on absorbent paper. Immediately, **wash the wells 4 times** with 300 ul of **1X wash buffer**. We recommend using an automated ELISA plate Washer for better consistency. Failure to wash the wells properly will lead to high blank or zero values. If washing manually, plate must be tapped over paper towel between washings to ensure proper washing.
4. Add **150 ul TMB substrate solution**. Mix gently for 5-10 seconds. Cover the plate and **incubate for 30 minutes** at room temp (no shaking necessary). Gently shake immediately before measuring the absorbance.
5. Stop the reaction by adding **50 ul of red stop solution** to all wells. Mix gently for 5-10 seconds.
6. Measure the absorbance at a **wavelength of 650 nm**. Wells should be read within 2 hours of stopping the reaction. Note: When Red Stop is used (supplied), it will result in a color ranging from a dark blue/purple to light pink based on the concentration of drug present. If 1 N HCl is preferred (not supplied), use 50 µL per well and read plate with a 450 nm filter. All QC data is generated without using a stopping reagent. Note: When acid stop is used, OD values will approximately double as compared to the OD values obtained with Red Stop.

NOTES

Read instructions carefully before the assay. Do not allow reagents to dry on the wells. Careful aspiration of the washing solution is essential for good assay precision. Since timing of the incubation steps is important to the performance of the assay, pipet the samples without interruption and it should not exceed 5 minutes to avoid assay drift. If more than one plate is being used in one run, it is recommended to include a standard curve on each plate. The unused strips should be stored in a sealed bag at 4°C. Do not touch the bottom of the wells.