TECHNICAL SUPPORT

For technical assistance, please contact our technical services department at 859/254-1221 or email at techservice-toxicology@neogen.com. Representatives are available Monday–Friday from 8:00 a.m.–6:00 p.m. EST.

INSERT TO BE USED WITH: Trenbolone

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Pink

DRUG DETECTION ELISA KIT

Trenbolone

Note changes: Kit Specific EIA Buffer

DRUG-ENZYME CONJUGATE FORMAT: CONCENTRATED
PACKAGE SIZE: SINGLE 96-WELL OR BULK 480-WELL

INTENDED USE

For the determination of trace quantities of drugs and/or other metabolites in equine or canine matrices.

STORAGE AND STABILITY

This kit can be used until the expiration date on the label when stored refrigerated at 4°C. Store controls at -20°C if not used within 10 days. **Note:** Some kits require controls to be stored frozen immediately upon receipt. Reference kit label for details.

MATERIALS PROVIDED

	SINGLE (96-WELL)	BULK (480-WELL)
EIA Buffer	40 mL Phosphate Buffer (ready-to-use) ***Kit Specific***	200 mL Phosphate Buffer (ready-to-use) ***Kit Specific***
Wash Buffer Concentrate (10X)	20 mL Phosphate Buffered saline solution. Dilute 1:10 with deionized water prior to use.	100 mL Phosphate Buffered saline solution. Dilute 1:10 with deionized water prior to use.
K-Blue Substrate	20 mL Tetramethylbenzidine (TMB) Light Sensitive (ready-to-use)	100 mL Tetramethylbenzidine (TMB) Light Sensitive (ready-to-use)
Drug-Enzyme Conjugate	200 µL drug-horseradish peroxidase conjugate. Dilute 1:180 before use.	1 mL drug-horseradish peroxidase conjugate. Dilute 1:180 before use.
Antibody Coated Plate	96-well Costar plate, in strips of 8 break-away wells. Coated with anti-drug antiserum. The plate is ready for use as is. **Do Not Wash**	(5) 96-well Costar plates, in strips of 8 break-away wells. Coated with anti-drug antiserum. The plate is ready for use as is. **Do Not Wash**
Qualitative Positive Control	750 µL provided (synthetic matrix) **Do Not Dilute**	Optional
Qualitative Negative Control	750 µL provided (synthetic matrix) **Do Not Dilute**	Optional

MATERIALS NEEDED BUT NOT PROVIDED

1. Deionized water
2. Precision pipettes that range from 10 µL – 1000 µL and disposable tips.
3. Graduated cylinder to dilute and mix wash buffer.
4. Plate cover or plastic film to cover plate during incubation.
5. Clean glassware (i.e. test tubes) to dilute samples.
6. Microplate reader with 650nm filter and optional Microplate shaker.

OPTIONAL MATERIALS

1. Neogen Red Stop
2. 1N HCl Acid

SAMPLE TREATMENT

Recommended minimum sample dilutions are referenced in the Neogen's Racing Drug Detection Manual. To request a copy, please contact your Neogen representative.

TEST PROCEDURES

The following test procedures can be run manually or on an automated instrument. Please contact your Neogen representative for assistance with protocols for automated instruments.

1. Determine the number of wells to be used.
2. Dilute the concentrated enzyme conjugate 1:180 with the EIA Buffer provided (i.e. 200 µL enzyme conjugate plus 35.8 mL EIA Buffer). Mix the solution by inversion. Do not vortex.
3. Add 20 µL of sample or control to the appropriate wells in duplicate. **Note:** DO NOT dilute Neogen's positive or negative controls.
4. Add 180 µL of diluted drug-enzyme conjugate to each well. Use 8-channel pipetter or 12-channel pipetter for rapid addition.
5. For manual runs, mix by gently shaking plate. A microplate shaker may be used.
6. Cover plate with plastic film or plate cover and incubate at room temperature for 45 minutes.
7. Dilute concentrated wash buffer 1:10 with deionized water (i.e. 20 mL of concentrated wash buffer plus 180 mL of deionized water). Mix thoroughly. Diluted wash buffer is stable for 5 days at room temperature or 7 days at 4°C.
8. Once the incubation is complete, dump the liquid from the wells. Tap the plate on a clean lint-free towel to remove any remaining liquid in the wells.
9. Wash each well with 300 µL of diluted wash buffer. Manual Wash: Repeat for a total of 3 washings, invert and tap dry the plate between each wash. After completing the last wash step wipe the bottom of the wells with a lint-free towel to remove any liquid on the outside of the wells. Automated Wash: Repeat for a total of 5 washings with 300 µL of diluted wash buffer. It is important for the automated washer to conduct a final aspirate cycle to eliminate residual amounts of wash buffer. Residual amounts of buffer in the wells will affect assay performance. NOTE: DI water should never be used for the plate wash.
10. Add 150 µL of the K-Blue Substrate to each well. For manual runs, use a multi-channel pipetter for best results. Mix by shaking plate gently.
11. Incubate at room temperature for 30 minutes.
12. Read results visually or with a microplate reader set at 650 nm. If a dual wavelength is used, set W_1 at 650 nm and W_2 at 490 nm. **Optional**: Add 50 µL of 1N HCl solution or Neogen Corporation's Red Stop Solution to each well to stop the enzyme reaction. When Red Stop Solution is used to stop the reaction, read plate at 650 nm. If 1N HCl solution is used to stop the reaction, read plate at 450 nm.

RESULTS INTERPRETATION

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.

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

Qualitative Controls: The Neogen positive and negative controls provided in the kit are intended 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. **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.