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.

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 am–6:00 pm EST.

COPYRIGHT

INSERT TO BE USED WITH: Clonidine/Romifidine, Detomidine, Fenspiride, Guanabenz, Nikethamide

944 Nandino Blvd., Lexington KY 40511 USA
800/477-8201 (USA/Canada) | 859/254-1221 | Fax: 859/255-5532
E-mail: inform@neogen.com | Web: NEOGEN.com

DRUG DETECTION ELISA KIT

DRUG-ENZYME CONJUGATE FORMAT: LYOPHILIZED
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 label when stored properly. Store lyophilized conjugate at -20ºC. Store controls at -20ºC if not used with 10 days. All other components should be stored at 2-8ºC. **Note:** Some kits require controls to be stored frozen immediately upon receipt. Reference kit label for details.

MATERIALS PROVIDED

DESCRIPTION	SINGLE (96-WELL)	BULK (480-WELL)
EIA Buffer	40 mL Phosphate Buffer (ready-to-use)	200 mL Phosphate Buffer (ready-to-use)
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: *a test kit will contain one of the following:*	a) **Drug-Enzyme Conjugate Lyophilized (excluding Guanabenz kit):** 2 x 100 µL Lyophilized vials of drug-horseradish peroxidase. Reconstitute with 100 µL of deionized water. a) **Drug-Enzyme Conjugate Lyophilized (Guanabenz kit only):** 3 x 75 µL Lyophilized vials of drug-horseradish peroxidase. Reconstitute with 75 uL of deionized water.	b) **Drug-Enzyme Conjugate Lyophilized (excluding Guanabenz kit):** 10 x 100 µL Lyophilized vials of drug-horseradish peroxidase. Reconstitute with 100 µL of deionized water. b) **Drug-Enzyme Conjugate Lyophilized (Guanabenz kit only):**15 x 75 µL Lyophilized vials of drug-horseradish peroxidase. Reconstitute with 75 µL of deionized water.
Antibody Coated Plate **Do Not Wash**	96-well Costar® plate, in strips of 8 break-away wells. Coated with anti-drug antiserum. The plate is ready for use as is.	(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.
Qualitative QC Positive Control	750 µL provided (synthetic matrix) **Do Not Dilute**	Optional
Qualitative QC 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 650 nm filter and optional Microplate shaker.

OPTIONAL MATERIALS

1. Neogen Red Stop
2. 1N HCl

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. To reconstitute the lyophilized conjugate, pipette 100 µL (75 µL for Guanabenz only) of deionized water into the vial. Allow conjugate to rehydrate by slowly rolling the vial. Invert the vial (tapping on the cap) to reconstitute any dry material trapped in the cap. Bring vial to an upright position and again tap the cap to release the volume from the cap. Do not vortex or shake. Avoid excess foaming. Continue to invert until all solid material is reconstituted. Allow the conjugate concentrate to incubate for the time listed in Table 1. The conjugate will reach full activity upon completion of this incubation. The shelf life of the reconstituted conjugate concentrate is also listed in Table 1. Label the vial with the date of reconstitution for future reference. Once vial is reconstituted, store at 2-8°C. Do not freeze. **Note: If more concentrated conjugate is needed than is contained in the first conjugate vial, reconstitute and use the second vial. Do not use the separate contents of vials in the same assay as some assay variability may result. If the contents of two or three vials are required of an assay, pool the concentrated conjugates. Use the expiration date of the oldest reconstituted vial for the pool. Alternatively, prepare the necessary volumes of diluted conjugate and pool before using the assay.**
2. Determine the number of wells to be used.
3. 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.
4. Add 20 µL of sample or control to the appropriate wells in duplicate. **Note:** DO NOT dilute Neogen's positive or negative controls.
5. Add 180 µL of diluted drug-enzyme conjugate to each well. Use 8-channel pipetter or 12-channel pipetter for rapid addition.
6. For manual runs, mix by gently shaking plate. A microplate shaker may be used.
7. Cover plate with plastic film or plate cover and incubate at room temperature. For incubation times, please reference the Certificate of Analysis provided with the kit.
8. 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 2-8°C.
9. 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.
10. 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. DI water should never be used for the plate wash.
11. 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.
12. Incubate at room temperature for 30 minutes.
13. 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.

TABLE 1

Kit Name	Incubation Time	Shelf Life at 4°C
Clonidine/Romifidine	15 minutes	14 days
Detomidine/Nikethamine	15 minutes	10 days
Fenspiride	15 minutes	7 days
Guanabenz	15 minutes	24 hours

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.