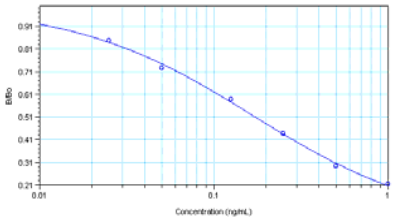
The Abraxis 19-Nortestosterone (Trenbolone) ELISA allows the determination of 41 samples in duplicate determination. Only a few grams or milliliters of sample are required. The test can be performed in less than 2 hours.

Performance Data

Test sensitivity: The limit of detection for 19-Nortestosterone, Serum = 1.0 ng/mL; Urine rapid screen : 1 ng/mL; Urine C18 method = 0.8 ng/mL; Tissue 2 ng/gm.

Standard Curve: The concentration of residue necessary to cause 50% inhibition (50% B/B_0) is approximately 0.19 ng/mL. Determinations closer to the middle of the calibration range of the test yield the most accurate results.

These values are used for demonstration purposes only; do not use these values for your determinations:



Test reproducibility: Coefficients of variation (CVs) for standards: <10%; CVs for samples: <15%.

Selectivity/ Cross-reactivity: This ELISA recognizes various 19-Nortestosterone with varying degrees:

19-Nortestosterone	100%
Trenbolone (17 ß)	85%
Trenbolone acetate	25%
Norehisterone	7.4%
19-Nor-4-Androstene,3,17 Dione	186%
19-Nortestosterone (17 ß)	50%
19-Nortestosterone (17 ß) Sulphate	40%
Boldenone	<0.2%
Progesterone	1.3%
Dihydrotestosterone	0.2%
Testosterone (17 ß)	0.8%
Pregnenolone	<0.1%

Samples: To eliminate matrix effects in a sample clean-up is required. See Preparation of Samples, section H.

General Limited Warranty: Abraxis LLC warrants the products manufactured by the Company against defects and workmanship when used in accordance with the applicable instructions for a period not to extend beyond the product's printed expiration date. **Abraxis makes no other warranty, expressed or implied. There is no warranty of merchantability or fitness for a particular purpose.**

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ABRAXIS

19-Nortestosterone ELISA, Microtiter Plate

Enzyme-Linked Immunosorbent Assay for the Determination of 19-Nortestosterone in Contaminated Samples

Product No. 52252R

1. General Description

The 19-Nortestosterone ELISA is an immunoassay for the detection of 19-Nortestosterone (Trenbolone). This test is suitable for the quantitative and/or qualitative detection of 19-Nortestosterone (Trenbolone) in contaminated samples including serum, urine, and tissue samples. Samples requiring regulatory action should be confirmed by HPLC, GC/MS, or other conventional methods.

2. Safety Instructions

The standard solutions in this test kit contain small amounts of 19-nortestosterone. In addition, the substrate solution contains tetramethylbenzidine and the stop solution contains diluted sulfuric acid. Avoid contact of stopping solution with skin and mucous membranes. If these reagents come in contact with the skin, wash with water.

3. Storage and Stability

The 19-Nortestosterone (Trenbolone) ELISA Kit should to be stored in the refrigerator (4–8°C). The solutions must be allowed to reach room temperature (20-25°C) before use. Reagents may be used until the expiration date on the box. The conjugate is supplied in a concentrated form and must be diluted before use (see Test Preparation section). Dilute only the amount needed for the samples to be run, as the diluted solution will only remain viable for one week (store refrigerated). Standards are provided in a lyophilized form, they need to be reconstituted with assay buffer and stored at 4–8°C for up to 30 days.

4. Test Principle

The test is a direct competitive ELISA based on the recognition of 19-Nortestosterone (Trenbolone) by specific antibodies. 19-Nortestosterones, when present in a sample, and a 19-Nortestosterone-enzyme conjugate compete for the binding sites of rabbit anti-19-Nortestosterone antibodies coated on the plate. After a washing step and addition of the substrate solution, a color signal is produced. The intensity of the blue color is inversely proportional to the concentration of 19-Nortestosterones present in the sample. The color reaction is stopped after a specified time and the color is evaluated using a microplate ELISA photometer. The concentrations of the samples are determined by interpolation using the standard curve constructed with each run.

5. Limitations of the 19-Nortestosterones ELISA, Possible Test Interference

Numerous organic and inorganic compounds commonly found in samples have been tested and found not to interfere with this test. However, due to the high variability of compounds that might be found in samples, test interferences caused by matrix effects can not be completely excluded. Mistakes in handling the test can also cause errors. Possible sources for such errors can be:

Inadequate storage conditions of the test kit, incorrect pipetting sequence or inaccurate volumes of the reagents, too long or too short incubation times during the immune and/or substrate reaction, extreme temperatures during the test performance (lower than 10°C or higher than 30°C).

The Abraxis 19-Nortestosterones ELISA kit provides screening results. As with any analytical technique (GC, HPLC, etc.), samples requiring regulatory action should be confirmed by an alternative method.

Working Instructions

A. Materials Provided

1. Microtiter plate coated with anti-19-Nortesterone.
2. 19-Nortestosterone Standards (7): 0, 0.11, 0.57, 0.97, 4.7, 9.3, and 23.7 ng/mL. Lyophilized, please refer to Test Preparation Section.
3. 19-Nortestosterone-HRP Conjugate, 1 vial (concentrated), please refer to Test Preparation Section.
4. Assay Buffer, 1 vial
5. DiluentWash Buffer (Ccncentrated), 1 vial. , please refer to Test Preparation Section.
6. Color (Substrate) Solution (TMB), 1 vial X 15 mL.
7. Stop Solution, I vial.

B. Test Preparation

Micro-pipetting equipment and pipette tips for pipetting the standards and the samples are necessary. We recommend using a multi-channel pipette or a stepping pipette for adding the conjugate, antibody, substrate and stop solutions in order to equalize the incubation periods of the solutions on the entire microtiter plate. Please use only the reagents and standards from one package lot in one test, as they have been adjusted in combination.

1. Adjust the microtiter plate and the reagents to room temperature before use.
2. Remove the number of microtiter plate strips required from the foil bag. The remaining strips are stored in the foil bag and zip-locked closed. Store the remaining kit in the refrigerator (4-8°C).
3. The standard solutions are supplied lyophilized. Reconstitute the contents of each bottle with 2.0 mL of Assay Buffer. Allow to stand for 30 minutes and mix by inversion before use. The standards are stable for at least 1 month when stored refrigerated.
4. The conjugate provided is in a concentrated form. Before each assay, calculate the volume of conjugate needed and dilute in 2 steps. Dilution 1. Add 20 uL of concentrated conjugate to 2.0 mL Assay buffer. Dilution 2. Add 50 uL dilution 1 to 4.5 mL of Assay Buffer. Once diluted, the conjugate solution will only remain viable for 1 day (sufficient for 1/3 of a microtiter plate). If additional samples are to be analyzed greater another day, a new vial of diluted conjugate must be prepared.
5. Dilute the wash buffer concentrate by adding 970 mL of deionized or distilled water. Store refrigerated.
6. Dilute the contents of the Assay Bufer with 60 mL of deionized or distilled water. Allow to stand for 30 minutes and gently shake to dissolve. Store refrigerated.
7. The stop solution should be handled with care as it contains diluted H_2SO_4.

C. Assay Procedure

1. Add 75 µL of Assay Buffer to each well.
2. Add 25 µL of the standard solutions and samples or sample extracts into the wells of the test strips according to the working scheme given. We recommend using duplicates or triplicates.
3. Add 25 µL of diluted enzyme conjugate solution to the individual wells successively using a multi-channel pipette or a stepping pipette. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Be careful not to spill contents.
4. Incubate the strips for 60 minutes at room temperature.
5. After incubation, remove the covering and vigorously shake the contents of these wells into a sink. Wash the strips six times using the 1X washing buffer solution. Use at least a volume of 250 µL of washing buffer for each well and each washing step. Remaining buffer in the wells should be removed by patting the plate dry on a stack of paper towels.
6. Add 125 µL of color (substrate) solution to the wells. Cover the wells with parafilm or tape and mix the contents by moving the strip holder in a circular motion on the benchtop for 30 seconds. Incubate the strips for 18-22 minutes at room temperature. Protect the strips from direct sunlight.
7. Add 100 µL of stop solution to the wells in the same sequence as for the substrate solution.
8. Read the absorbance at 450 nm using a microplate ELISA photometer within 10 minutes after the addition of the stopping solution.

D. Evaluation

The evaluation of the ELISA can be performed using commercial ELISA evaluation programs (4-Parameter (preferred) or Logit/Log). For manual evaluation, calculate the mean absorbance value for each of the standards. Calculate the $\%B/B_0$ for each standard by dividing the mean absorbance value for each standard by the Zero Standard (Standard 0) mean absorbance. Construct a standard curve by plotting the $\%B/B_0$ for each standard on the vertical linear (y) axis versus the corresponding 19-Nortestosterones concentration on the horizontal logarithmic (x) axis on graph paper. $\%B/B_0$ for samples will then yield levels in ppb of 19-Nortestosterones by interpolation using the standard curve. Samples showing a higher concentration than Standard 6 (23.7 ng/mL) must be diluted further to obtain accurate results. Appropriate dilution factor should be applied (multiplied to obtain final concentration).

E. Additional Materials (not included with the test kit)

1. Micro-pipettes with disposable plastic tips (10-200 and 200-1000 µL)
2. Multi-channel pipette (10-250 µL) or stepper pipette with plastic tips (10-250 µL)
3. Microtiter plate reader (wave length 450 nm)
4. Timer, Vortexer, Heating Block, fume hood
5. Tape or Parafilm
6. Tert-Butyl methyl ether (HPLC grade), Sodium acetate, ß-glucoronidase, Methanol, Trizma base, C18 columns (Baker 7020-01)
7. Vacuum manifold for C18 extraction

F. Working Scheme

The microtiter plate consists of 12 strips of 8 wells, which can be used individually for the test. The standards must be run with each test. Never use the values of standards which have been determined in a test performed previously.

Std 0-Std 6: Standards
0; 0.11; 0.57; 0.97; 4.7; 9.3; 23.7 ppb

Samp1, Samp2, etc.: Samples

	1	2	3	4	5	6	7	8	9	10	11	12
A	Std 0	Std 4	Samp2									
B	Std 0	Std 4	Samp2									
C	Std 1	Std 5	etc.									
D	Std 1	Std 5	etc.									
E	Std 2	Std 6										
F	Std 2	Std 6										
G	Std 3	Samp1										
H	Std 3	Samp1										

G. Preparation of Samples

Urine Sample-Rapid Screen-Method A

1. Centrifuge urine sample at 4000 rpm for 10 minutes.
2. Dilute centrifuged urine 10 fold in assay buffer (e.g. 50 µL urine + 450 µL assay buffer). Sample is now ready to be analyzed in the ELISA.

The 19-Nortestosterones concentration in the sample is determined by multiplying the ELISA results of the prepared sample by 10.

Urine Sample C18 Extraction-Method B

1. Centrifuge urine sample at 4000 rpm for 10 minutes.
2. Add 0.5 mL of centrifuged urine to 3.0 mL of 50 mM sodium acetate buffer, pH 4.8 and 1,000 units of ß-glucoronidase (Helix pomatia, Sigma G-0876). Vortex thoroughly and incubate for 3 hours at 37 °C.
3. Rinse C18 column with 2.0 mL of 100% MeOH. Followed by 2.0 mL of Trizma Buffer containing 20% MeOH, pH 8.5.
4. Apply sample form step 2.
5. Rinse column with 2.0 mL of Trizma Buffer containing 20% MeOH, pH 8.5. Followed by a rinse with 3.0 mL of 50% MeOH/50% water.
4. Elute with 1.0 mL of 80% MeOH/20% water into a clean glass tube.
5. Evaporate eluant to dryness at 65 °C using a heating block with air stream. Resuspend in 0.5 mL of Assay buffer and analyze in the ELISA. No multiplication factor is needed (no dilution factor), the concentration of 19-Nortestosterone in the sample is readed directly.

Serum Sample Preparation

1. To 0.5 mL of serum sample, add 5 mL of tertButyl methyl ether in a glass test tube. Vortex for 3 minutes.
2. Let serum/TBME mixture to settle for 20 minutes. Transfer 4 mL of the TBME layer into a glass tube.
3. Evaporate TBME to dryness at 50 °C. Resuspend in 0.4 mL of Assay Bufer. Vortex for 3 minutes and analyze in the ELISA. The 19-Nortestosterone concentration in the sample is determined by Obtaining the ELISA results directly from the assay. No multiplication factor is needed.

Please contact company for the analysis of tissue samples for additional clean-up procedures.