Expected values

Four urine samples from various species were tested in the assay. Neat concentrations of 11-ketotestosterone ranged from 1.162 ng/mL to 6.576 ng/mL. When adjusted for urine creatine using the Urinary Creatinine Detection kit, EIACUN, the values ranged from 7.1 ng/mL to 11.8 ng/mg creatinine.

Fecal samples from four different species were extracted and tested in the assay. Adjusted neat concentrations of 11-ketotestosterone ranged from 28.5 pg/mg to 359.3 pg/mg dried feces.

Extracted serum from different species were also tested in the assay. Neat concentrations of 11-ketotestosterone in extracted human serum ranged from 144.5 pg/mL to 449.3 pg/mL. Extracted serum from white sturgeon fish ranged from 219.02 pg/mL to 28,657.8 pg/mL.

Sensitivity

The analytical sensitivity of the assay is 1.85 pg/mL. This was determined by adding two standard deviations to the mean O.D. obtained when the zero and Std7 were assayed 20 times and calculating the corresponding concentration.

Linearity

Linearity was determined by taking two urine samples diluted with Assay Buffer, one with a low diluted 11-ketotestosterone level of 77.9 pg/mL and one with a higher diluted level of 539.6 pg/mL, mixed in the ratios give below. The measured concentrations were compared to the expected values based on the ratios used.

High Urine (%)	Low Urine (%)	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	Recovery (%)
80	20	447.3	446.3	99.8
60	40	354.9	377.8	106.4
40	60	247.0	290.2	117.5
20	80	170.2	199.2	117.0
			Mean Recovery	**110.2%**

Specificity

The following cross reactants were tested in the assay and calculate at the 50% binding point.

Steroid	Cross-reactivity (%)
11-Ketotestosterone	100
Testosterone	2.03
11-Ketoandrostenedione	1.70
Dehydroandrosterone	1.48
DHEA	0.95
Progesterone	0.24
17-Hydroxyprogesterone	0.20
Epiandrosterone	0.12
Androsterone	0.08
17β-Estradiol	<0.05

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

REF Catalog Number | LOT Batch code | Temperature limitation | Use by | Manufacturer | Consult instructions for use | Caution, consult accompanying documents

Manufacturer: Life Technologies Corporation | 7335 Executive Way | Frederick, MD 21704 | USA

11-Ketotestosterone Competitive ELISA Kit

Catalog Number EIA11K (96 tests), EIA11KX10 (10 x 96 tests)

Rev A.0

Note: For safety and biohazard guidelines, see the "Safety" appendix in the *ELISA Technical Guide* (Pub. no. MAN0006706). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Product description

The 11-Ketotestosterone ELISA Kit is a solid-phase monoclonal, antibody-based, competitive Enzyme-Linked Immunosorbent Assay (ELISA). This assay is designed to detect and quantify the level of 11-Ketotestosterone in extracted serum, plasma, and dried fecal or urine samples. The assay recognizes 11-Ketotestosterone independent of species.

Contents and storage

Kit and components are shipped at –20°C. Upon receipt, store the kit at –20°C. Once open, store the kit at 4°C and use within 2 weeks.

Components	Quantity (96 tests)	Quantity (10 x 96 tests)
Coated Clear 96 Well Plates: plastic microtiter plate coated with goat anti-rabbit IgG	1 plate	10 plates
11-Ketotestosterone Standard; 100,000 pg/mL	70 µL	10 x 70 µL
11-Ketotestosterone Antibody	3 mL	10 x 3 mL
11-Ketotestosterone Conjugate	3 mL	10 x 3 mL
Assay Buffer Concentrate (5X)	28 mL	10 x 28 mL
Wash Buffer Concentrate (20X)	30 mL	2 x 125 mL
TMB (Tetramethylbenzidine) Substrate	11 mL	10 x 11 mL
Stop Solution; contains 1 M HCl, **CAUSTIC**	5 mL	50 mL
Plate Sealer	1	10

Materials required but not supplied

- Distilled or deionized water
- Microtiter plate reader with software capable of measurement at 450 nm
- Calibrated adjustable precision pipettes and glass or plastic tubes for diluting solution
- Diethyl ether or ethyl acetate for extraction of serum and plasma
- Ethanol for extraction of fecal material
- Plate washer: automated or manual (squirt bottle, manifold dispenser, or equivalent)

Procedural guidelines

IMPORTANT! Reagents are lot-specific. Do not mix or interchange different reagent lots from various kit lots.

Prepare 1X Wash Buffer

1. Dilute 15 mL of Wash Buffer Concentrate (20 mL) by adding 1 part of the Concentrate to 19 parts of deionized or distilled water. Label as 1X Wash Buffer.
2. Store the concentrate and 1X Wash Buffer in the refrigerator. Use the diluted buffer within 3 months.

Prepare 1X Assay Buffer

1. Dilute 14 mL of Assay Buffer Concentrate (5X) with 56 mL of deionized or distilled water. Label as 1X Assay Buffer.
2. Store the concentrate and 1X Assay Buffer in the refrigerator. The 1X Assay Buffer is stable at 4°C for 3 months.

For Research Use Only. Not for use in diagnostic procedures.

Sample preparation guidelines

- Refer to the *ELISA Technical Guide* at **thermofisher.com** for detailed sample preparation procedures.
- Collect samples in pyrogen/endotoxin-free tubes.
- Freeze samples after collection if samples will not be tested immediately. Avoid multiple freeze-thaw cycles of frozen samples. Thaw completely and mix well (do not vortex) prior to analysis.
- Avoid the use of hemolyzed or lipemic sera.
- If large amounts of particulate matter are present in the sample, centrifuge or filter sample prior to analysis.

Prepare samples

Sample concentrations should be within the range of the standard curve. Because conditions may vary, each investigator should determine the optimal dilution for each application.

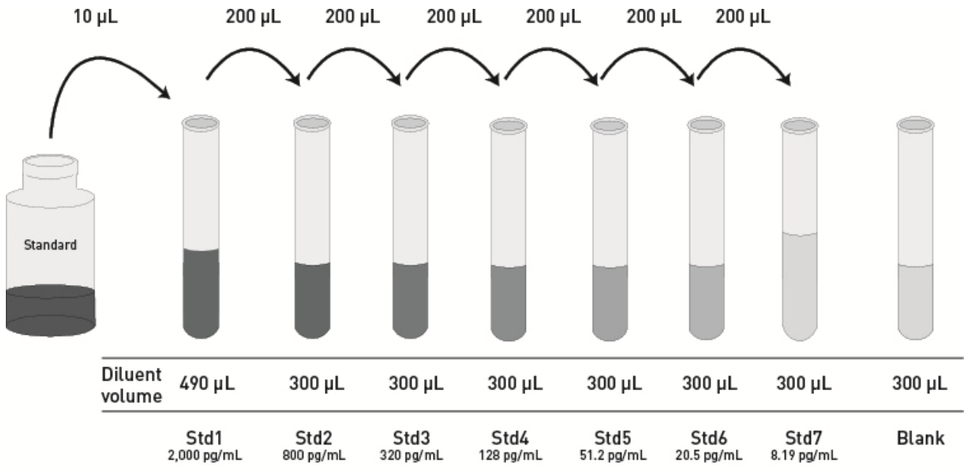
Use all samples within **2 hours** of dilution.

Sample type	Procedure
Serum and plasma samples	1. Add diethyl ether or ethyl acetate to samples at a 5:1 (v/v) solvent:sample ratio. 2. Mix solutions by vortexing for 2 minutes. Allow solvent layer to separate for 5 minutes. 3. Freeze samples in a dry ice/ethanol bath and pipette off the solvent solution from the top of the sample into a clean tube. Repeat steps 1-3 for maximum extraction efficiency, combining top layer of solvent solutions. 4. Dry pooled solvent samples down in a Speedvac for 2-3 hrs. If samples need to be stored, they should be kept at -20°C. 5. Redissolve samples at room temperature in 1X Assay Buffer. A minimum of 125 µL of the Assay Buffer is recommended for reconstitution to allow for duplicate sample measurement.
Urine	Dilute samples 1:8 with 1X Assay Buffer. **Note**: A Urinary Creatinine Detection Kit (Cat. No. EIACUN) is available for measuring urine creatinine for normalization of 11-Ketotestosterone in a random urine specimen.
Dried fecal samples	See detailed extraction protocol on the product page at **thermofisher.com.** **Note:** The ethanol concentration in the final diluted Assay Buffer dilution added to the well should be < 5%.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

1. Add 10 µL 11-Ketotestosterone Standard to one tube containing 490 µL 1X Assay Buffer and label as 2,000 pg/mL 11-Ketotestosterone.
2. Add 300 µL 1X Assay Buffer to each of 6 tubes labeled as follows: 800, 320, 128, 51.2, 20.5, and 8.19 pg/mL 11-Ketotestosterone.
3. Take 200 µL of the solution in tube #1 and add it to tube #2. Vortex completely. Repeat the serial dilutions for tubes #3 through #8 as shown in the figure below. Mix thoroughly between steps.
4. **Use the standards within 2 hours of preparation**.



Perform ELISA (Total assay time: 2.5 hours)

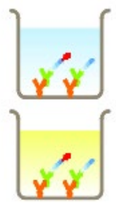
IMPORTANT! Perform a standard curve with each assay.

Allow all components to reach room temperature before use.

Mix all liquid reagents prior to use. Determine the number of 8-well strips required for the assay. Insert the strips in the frames for use. Re-bag any unused strips and frames, and store desiccated at 2°C to 8°C for future use. The silica pack in the bag keeps the plate dry and will turn from blue to pink if the bag is not properly sealed.

Bind antigen

a. Add 50 µL of standards or samples (see "Prepare samples" on page 2) to the appropriate wells.
b. Add 75 µL of 1X Assay Buffer into wells for detecting non-specific binding (NSB).
c. Add 50 µL of 1X Assay Buffer into maximum binding wells (B0 or Zero standard).
d. Add 25 µL of 11-Ketotestosterone Conjugate to each well.
e. Add 25 µL of 11-Ketotestosterone Antibody to each well except NSB wells.
f. Tap the side of the plate to mix. Cover the plate with plate sealer.
g. Shake at room temperature for 2 hours. If the plate is not shaken, signal bounds will be ~ 20% lower.
h. Thoroughly aspirate the solution and wash wells 4 times with 300 µL of 1X Wash Buffer.



Add chromogen.

a. Add 100 µL TMB Substrate to each well. The substrate solution will begin to turn blue.
b. Incubate for 30 minutes at room temperature without shaking.
Note: TMB should not touch aluminum foil or other metals.

Add stop solution

Add 50 µL Stop Solution to each well. Tap side of the plate gently to mix. The solution in the wells will change from blue to yellow.

Read the plate and generate the standard curve

1. Read the absorbance at 450 nm. Read the plate within 10 minutes after adding the Stop Solution.
2. Average the duplicate Optical Density (OD) values for each standard and sample. Use curve-fitting software to generate the standard curve. A four-parameter algorithm provides the best standard curve fit. Optimally, the background absorbance may be subtracted from all data points, including standards, unknowns, and controls, prior to plotting.
3. Calculate the concentrations for unknown samples and controls from the %B/B0 curve. Multiply value(s) obtained for sample(s) by the appropriate factor to correct for the sample dilution.
Note: Dilute samples producing signals lower than that of the highest standard in 1X Assay Buffer and reanalyze. Multiply the concentration by the appropriate dilution factor.

Performance characteristics

Standard curve (example)

The following data were obtained for the various standards over the range of 0–2,000 pg/mL 11-Ketotestosterone.

Std 11-Ketotestosterone (pg/mL)	Mean OD
2000	0.200
800	0.293
320	0.441
128	0.641
51.2	0.850
20.5	1.004
8.19	1.068
0	1.142

Conversion Factor: 100 pg/mL of 11-ketotestosterone is equivalent to 330.7 pM.

Intra-assay precision

Samples were assayed in replicates of 20 to determine precision within an assay.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	349.5	262.3	184.0
CV (%)	4.6	6.8	6.6

CV = Coefficient of Variation

Inter-assay precision

Samples were assayed in duplicates in 19 assay runs by three operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	407.4	299.6	206.4
CV (%)	8.2	6.5	8.0

CV = Coefficient of Variation