Performance characteristics, continued

Expected values

Sixteen serum and four plasma samples from humans were tested in the assay at various dilutions. Adjusted neat concentrations of testosterone ranges from 317.0 pg/mL to 8,440.5 pg/mL. Seven urine samples from various species were tested in the assay. Adjusted neat concentrations of testosterone ranged from 51.2 pg/mL to 6157.6 pg/mL. Fecal samples from four different species were extracted and tested in the assay. Adjusted neat concentrations of testosterone ranged from 22.24 pg/mL to 149.92 pg/mL dried feces.

Note – Each lab should establish its own range. This is to serve as a guide only.

Sensitivity

The analytical sensitivity of testosterone is 2.97 pg/mL. This was determined by adding two standard deviations to the mean OD obtained when the zero and Std8 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 testosterone level of 27.3 pg/mL and one with a high diluted testosterone level of 253.0 pg/mL, and mixing them in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

Fecal Extract

High Sample (%)	Low Sample (%)	Expected Conc. (pg/mL)	Observed Conc. (pg/mL)	Recovery (%)
80	20	207.86	210.5	101.3
60	40	162.72	178.5	109.7
40	60	117.58	133.5	113.5
20	80	72.44	88.8	122.6
			Mean Recovery	**111.8%**

Specificity

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

Steroid	Cross-reactivity (%)
Testosterone	100
11-Ketotestosterone	14.64
Dihydrotestosterone	5.02
Estradiol	4.34
DHEA	0.030
Cholesterol	<0.01
Aldosterone	<0.01
Corticosterone	<0.01
Progesterone	<0.01
Pregnenolone	<0.01
Cortisone	<0.01
Cortisol	<0.01

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

The information in this guide is subject to change without notice.

Testosterone Competitive ELISA Kit

Catalog Number EIATES (96 tests), EIATESX10 (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 Testosterone 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 testosterone in saliva, serum, plasma, and dried fecal samples, or urine. The assay recognizes testosterone 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 Plate: plastic microtiter plate coated with donkey anti-sheep IgG	1 plate	10 plates
Testosterone Standard: 200,000 pg/mL	70 µL	10 x 70 µL
Testosterone Antibody	3 mL	10 x 3 mL
Testosterone 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	1 x 50 mL
Dissociation Reagent: to be used only with serum and plasma samples	1 mL	10 x 1 mL
Plate Sealer	1	1

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
- Ethanol or ethyl acetate for extraction of fecal samples
- 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 (20X) with 285 mL 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.

ThermoFisher SCIENTIFIC

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.

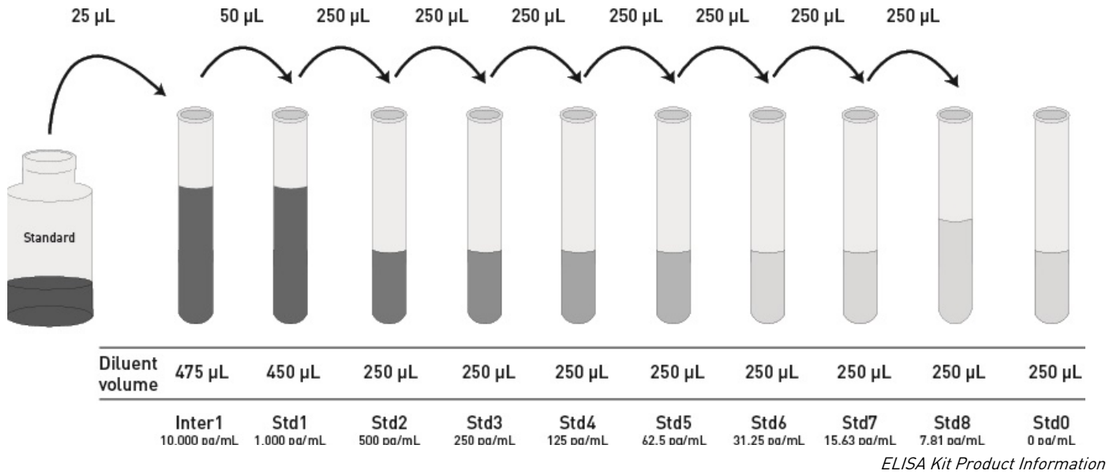
Sample type	Procedure
Serum and plasma	**Note: Dissociation Reagent is to be used only with Serum and Plasma samples.** 1. Allow the Dissociation Reagent to warm completely to room temperature before use. 2. Pipette 10 µL of Dissociation Reagent into a 1 mL tube. 3. Add 10 µL of serum or plasma to the Dissociation Reagent in the tube. Vortex periodically while incubating at room temperature for 15 minutes or longer. 4. Dilute this 1:2 mixture of serum or plasma and dissociation reagent to at least 1:18 with 1X Assay Buffer before running the assay. The final serum and plasma dilutions should be ≥ 1:36. This is the minimum dilution to remove matrix effects of the Dissociation Reagent in the assay.
Urine	Dilute samples ≥1:8 with 1X Assay Buffer. Most samples will require significant diluent to fall within the standard range. **Note**: For comparison to creatinine as a urine volume marker, please see our NIST-calibrated Urinary Creatinine Detection Kit (Cat. No. EIACUN). Use all samples within 2 hours of preparation.
Dried feces	See detailed extraction protocol on the product page at **thermofisher.com**
Saliva	Saliva samples should be diluted ≥ 1:4 in 1X Assay Buffer prior to running in the assay.

Dilute standards

Note: Use glass or plastic tubes for diluting standards.

The Testosterone Standard contains an organic solvent. Pipette the standard up and down several times to wet the pipet tip before transfer to ensure that volumes are accurate.

1. Add 25 µL Testosterone Standard to one tube containing 475 µL 1X Assay Buffer and label as 10,000 pg/mL.
2. Add 450 µL 1X Assay Buffer to the first of 8 tubes and label as 1,000 pg/mL.
3. Add 250 µL 1X Assay Buffer to each of the remaining 7 tubes and label as follows: 500, 250, 125, 62.5, 31.25, 15.63, and 7.81 pg/mL.
4. Take 250 µL of the testosterone solution in Std. tube #1 and add it to Std tube #2. Vortex completely. Continue to serially dilute tubes #3 through #8. Mix thoroughly between steps.
5. **Use the standards within 2 hours of preparation**.



Perform ELISA (Total assay time: 2.5 hours)

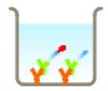
IMPORTANT! Perform a standard curve with each assay.

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 Testosterone Conjugate to each well.
e. Add 25 µL of Testosterone 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 signals bound 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 0–1,000 pg/mL testosterone.

Std Testosterone (pg/mL)	Mean OD
1,000	0.189
500	0.265
250	0.406
125	0.617
62.5	0.875
31.25	1.078
15.63	1.245
7.81	1.294
0	1.376

Conversion Factor: 100 pg/mL of testosterone is equivalent to 346.7 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)	151.5	74.8	36.9
CV (%)	4.1	4.9	7.0

CV = Coefficient of Variation

Inter-assay precision

Samples were assayed in duplicates in 24 assay runs by six operators to determine precision between assays.

Parameters	Sample 1	Sample 2	Sample 3
Mean (pg/mL)	206.4	151.7	75.0
CV (%)	7.5	10.9	7.3

CV = Coefficient of Variation