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Testosterone competitive ELISA kit datasheet

For the quantitative detection of Testosterone in serum, plasma and urine.

General information

Catalogue Number	KE30009
Product Name	Testosterone ELISA Kit
Species cross-reactivity	Testosterone
Range (calibration Range)	0.781-50 ng/mL
Tested applications	Competitive ELISA

Database links

CAS	58-22-0

Kit components & storage

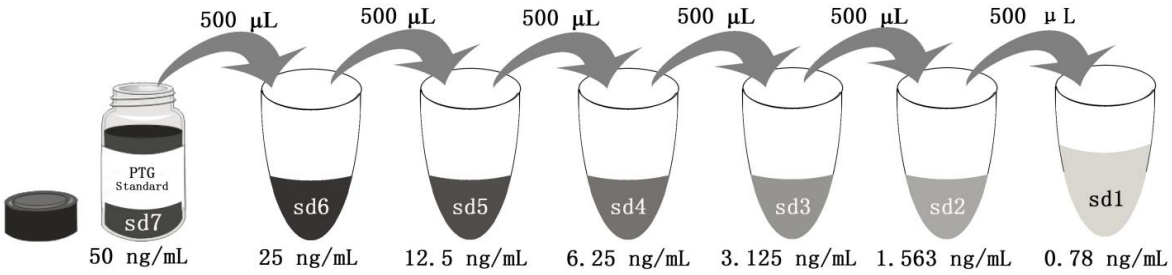
Microplate - antigen coated 96-well Microplate (8 well × 12 strips)	1 plate	Store at 2-8°C for six months
Standard - 100 ng/bottle; lyophilized*	2 bottles	Store at 2-8°C for six months
Detection antibody, HRP-conjugated (100X) - 60 µL/vial	1 vial	Store at 2-8°C for six months
Sample Diluent PT 4B2 - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Detection Diluent - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Wash Buffer Concentrate (20X) - 30 mL/bottle	1 bottle	Store at 2-8°C for six months
Tetramethylbenzidine Substrate (TMB) - 12 mL/bottle	1 bottle	Store at 2-8°C for six months
Stop Solution - 12 mL/bottle	1 bottle	Store at 2-8°C for six months
Plate Cover Seals	4 pieces	

NB: Do not use the kit after the expiration date.

Sample Diluent PT 4B2 is for standard, human serum, plasma and urine samples.

Detection Diluent is for Detection antibody.

*Add **2 mL** Sample Diluent PT 4B2 in standard. This reconstitution gives a stock solution of 50 ng/mL.



Add # µL of Standard diluted in the previous step	—	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
# µL of Sample Diluent PT 4B2	**2000 µL**	500 µL	500 µL	500 µL	500 µL	500 µL	500 µL
	"sd7"	"sd6"	"sd5"	"sd4"	"sd3"	"sd2"	"sd1"

Product description

KE30009 is a vitro competitive binding enzyme-linked immunosorbent assay（Competitive ELISA）. The testosterone ELISA kit is to be used to detect and quantify protein levels of vitro human testosterone. The assay recognizes human testosterone. A 96-well plate has been precoated with Testosterone antigen. Samples and the antibody-HRP conjugate are added to the wells, where testosterone in the sample competes with the testosterone antigen immobilized on the microplate for binding with the antibody-HRP. After incubation, the wells are washed to remove unbound material and TMB substrate is then added which is catalyzed by HRP to produce blue coloration. The reaction is terminated by addition of Stop Solution which stops the color development and produces a color change from blue to yellow. The intensity of signal is inversely proportional to the amount of Testosterone in the sample and the intensity is measured at 450 nm with the correction wavelength set at 630 nm.

Background

Testosterone is the primary male sex hormone (androgen) and is crucial for the development of male reproductive tissues, as well as for promoting secondary sexual characteristics. While it is produced in much larger amounts in men, it is also an important hormone for women, playing a key role in libido, bone strength, and muscle maintenance.

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Sample preparation

1:2 or 1:4 dilution is recommended for human serum, plasma and urine samples.

Safety notes

This product is sold for lab research and development use ONLY and not for use in humans or animals.
Avoid any skin and eye contact with Stop Solution and TMB. In case of contact, wash thoroughly with water.

Assay procedure summary

Add 50 µL standard or sample to appropriate wells

⇩

Add 50 µL antibody-HRP solution (1x) to each well

⇩

Incubate the plate for 1 hour at 37℃

⇩

Aspirate and wash each well four times with 400 µL 1x Wash Buffer

⇩

Add 100 µL TMB substrate solution to each well

⇩

Incubate the plate for 15 minutes at 37℃

⇩

Add 100 µL Stop solution to each well and Read OD immediately

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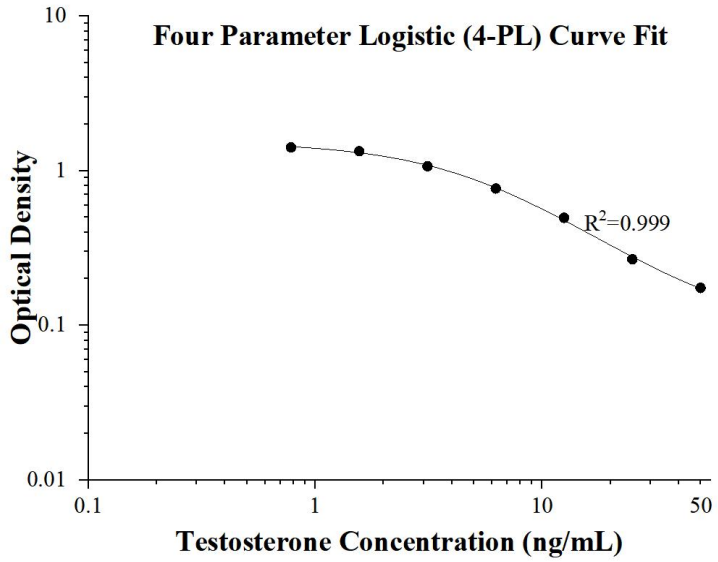
Typical data

These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(ng/mL)	O.D	Average
0	1.795	1.7948
	1.7945	
0.78	1.4265	1.4162
	1.4058	
1.563	1.3223	1.3371
	1.3518	
3.125	1.06	1.0690
	1.078	
6.25	0.7737	0.7673
	0.7608	
12.5	0.4961	0.4958
	0.4954	
2.5	0.2724	0.2673
	0.2621	
50	0.1728	0.1746
	0.1763	

Precision

Intra-assay Precision (Precision within an assay) Three samples of known concentration were tested 20 times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays) Three samples of known concentration were tested in 24 separate assays to assess inter-assay precision.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	8	8	8	16	16	16
Mean (ng/mL)	24.69	5.87	2.77	25.95	6.21	2.96
SD	1.51	0.43	0.24	1.99	0.60	0.32
CV%	6.12	7.33	8.66	7.67	9.66	10.81

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Recovery

The recovery of Testosterone spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Sample Type		Average% of Expected	Range (%)
Human serum	1:2	77	71-82
	1:4	80	71-90
Urine	1:2	95	82-105
	1:4	93	85-102

Sample values

Human serum/Urine - Human serum and urine samples were evaluated for the presence of Testosterone in this assay.

Sample Type	Mean (ng/mL)	Range (ng/mL)
Human serum (n=16)	5.99	2.12-8.53
Urine (n=8)	37.97	3.87-91.50

Sensitivity

The minimum detectable dose of Testosterone is 0.01 ng/mL. This was determined by adding two standard deviations to the concentration corresponding to the mean O.D. of 20 zero standard replicates.

Linearity

To assess the linearity of the assay, human serum and urine samples were diluted with the appropriate **Sample Diluent** to produce samples with values within the dynamic range of the assay.

		Human serum	Urine
1:2	Average% of Expected	100	100
	Range (%)	-	-
1:4	Average% of Expected	88	93
	Range (%)	81-95	85-102
1:8	Average% of Expected	102	100
	Range (%)	101-103	87-113
1:16	Average% of Expected	102	99
	Range (%)	98-106	86-112

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Specificity

This assay recognizes natural and recombinant Testosterone.

The following factors prepared at 100 ng/mL were assayed and exhibited no cross-reactivity or interference.

Recombinant human:

Progesterone

SHBG

References

1. Hochu, Gabrielle et al. Translational andrology and urology vol. 14,12 (2025): 3975-3987.
2. Feller, Elaine K et al. AIMS neuroscience vol. 12,4 614-634. 26 Nov. 2025.
3. Cooke, B A et al. The Biochemical journal vol. 160,3 (1976): 439-46.