

ab283979 – Dihydrotestosterone ELISA Kit

For the quantitative measurement of Dihydrotestosterone (DHT) in plasma, serum, milk, urine and saliva samples.

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab283979

Storage and Stability: Store kit at +4°C immediately upon receipt, apart from the Standard and SP Conjugate which should be stored at -20°C. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Materials Supplied

Item	Quantity	Storage Condition
100X Streptavidin-Peroxidase Conjugate	80 µL	-20°C
10X Diluent M Concentrate	20 mL	4°C
3X Biotinylated Dihydrotestosterone Steroid	1 vial	4°C
20X Wash Buffer Concentrate	30 mL	4°C
Chromogen Substrate	7 mL	4°C
Dihydrotestosterone Microplate	96 wells	4°C
Dihydrotestosterone Standard	1 vial	-20°C
Sealing Tapes	3 units	N/A
Stop Solution	11 mL	4°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

Microplate reader capable of measuring absorbance at 450 nm

Pipettes (1-20 µL, 20-200 µL, 200-1000 µL, and multiple channel)

Deionized or distilled reagent grade water

Reagents Preparation

Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.

Prepare only as much reagent as is needed on the day of the experiment.

If crystals have formed in the concentrate, mix gently until the crystals have completely dissolved.

Δ Note: Concentration of the kit components are lot-specific, and the end user should always refer to the vial label.

10X Diluent M Concentrate: Dilute the Diluent M Concentrate 1:10 with reagent grade water to produce a 1x solution. When diluting the concentrate, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle. Store for up to 30 days at 4°C.

3X Biotinylated Dihydrotestosterone Steroid: Reconstitute the Biotinylated Dihydrotestosterone Steroid with 4 mL Diluent M to generate a stock solution. Allow the vial to sit for 10 minutes with gentle agitation prior to use. From the stock solution, dilute 1:3 with Diluent M to produce a 1X working solution. Any remaining stock solution should be stored at -20°C and used within 30 days. Avoid repeated freeze-thaw cycles.

20X Wash Buffer Concentrate: Dilute the Wash Buffer Concentrate 1:20 with reagent grade water to produce a 1x solution. When diluting the concentrate, make sure to rinse the bottle thoroughly to extract any precipitates left in the bottle.

100X Streptavidin-Peroxidase Conjugate: Spin down the SP Conjugate briefly and dilute the desired amount of the conjugate 1:100 with Diluent M to produce a 1x solution. The undiluted conjugate should be stored at -20°C.

Standard Preparation

- Allow the Dihydrotestosterone Standard vial to warm to room temperature with gentle agitation prior to making dilutions.
- Label tubes 1-6.
- Prepare duplicate or triplicate standard points by serially diluting from the supplied standard stock solution (16 ng/ml) 4-fold with Diluent M to produce 4, 1, 0.25, 0.063, and 0.016063 ng/ml solutions.
- Diluent M (tube #6) serves as the zero standard (0 ng/ml).
- Aliquot remaining stock solution to limit repeated freeze-thaw cycles. This solution should be stored at -20°C.

Standard #	Volume to dilute (µL)	Volume 1X Diluent M (µL)	DHT (ng/ml)
1	120µl	-	16
2	120 µL Standard #1	360	4
3	120 µL Standard #2	360	1
4	120 µL Standard #3	360	0.25
5	120 µL Standard #4	360	0.063
6	-	360	0.00

Sample Preparation

Plasma: Collect plasma using one-tenth volume of 0.1 M sodium citrate as an anticoagulant. Centrifuge samples at 3000 x g for 10 minutes and collect plasma.

A human plasma sample is suggested for use at 1x; however, user should determine optimal dilution factor depending on application needs.

The undiluted samples can be stored at -20°C or below for up to 3 months.

Avoid repeated freeze-thaw cycles (EDTA or Heparin can also be used as an anticoagulant).

Serum: Samples should be collected into a serum separator tube.

After clot formation, centrifuge samples at 3000 x g for 10 minutes and remove serum.

A human serum sample is suggested for use at 1x; however, user should determine optimal dilution factor depending on application needs.

The undiluted samples can be stored at -20°C or below for up to 3 months.

Avoid repeated freeze-thaw cycles.

Milk: Collect human milk using sample tube.

Centrifuge samples at 800 x g for 10 minutes. A 1:4 sample dilution is suggested into Diluent M; however, user should determine optimal dilution factor depending on application needs.

The undiluted samples can be stored at -20°C or below for up to 3 months.

Avoid repeated freeze-thaw cycles.

Urine: Collect urine using sample pot.

Centrifuge samples at 800 x g for 10 minutes.

A human urine sample is suggested for use at 1x or within the range of 2x – 40x into Diluent M; however, user should determine optimal dilution factor depending on application needs.

The undiluted samples can be stored at -20°C or below for up to 3 months.

Avoid repeated freeze-thaw cycles.

Saliva: Collect human saliva using samples tube.

Centrifuge samples at 800 x g for 10 minutes.

The sample is suggested for use at 1x or within the range of 2x – 10x into Diluent M; however, user should determine optimal dilution factor depending on application needs.

The undiluted samples can be stored at -20°C or below for up to 3 months.

Avoid repeated freeze-thaw cycles.

Plate Preparation

The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents.

Unused plate strips should be immediately returned to the foil pouch containing the desiccant pack, resealed and stored at 4°C.

For each assay performed, a minimum of two wells must be used as the zero control.

For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

Assay Procedure

Equilibrate all materials and prepared reagents to room temperature prior to use.

We recommend that you assay all standards, controls, and samples in duplicate

1. Add 25 µl of Dihydrotestosterone Standard or sample to each well, and immediately add 25 µl of Biotinylated Dihydrotestosterone Steroid to each well (on top of the standard or sample). Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 2 hours. Start the timer after the last addition.
2. Wash the microplate manually or automatically using a microplate washer. Invert the plate and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If washing manually, wash five times with 200 µl of Wash Buffer per well. Invert the plate each time and decant the contents; hit 4-5 times on absorbent material to completely remove the liquid. If using a microplate washer, wash six times with 300 µl of Wash Buffer per well; invert the plate and hit 4-5 times on absorbent material to completely remove the liquid.
3. Add 50 µl of SP Conjugate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Cover wells with a sealing tape and incubate for 30 minutes. Turn on the microplate reader and set up the program in advance.
4. Wash the microplate as described above.
5. Add 50 µl of Chromogen Substrate to each well. Gently tap plate to thoroughly coat the wells. Break any bubbles that may have formed. Incubate in ambient light for 10 minutes or until the optimal blue colour density develops.
6. Add 50 µl of Stop Solution to each well. The colour will change from blue to yellow. Gently tap plate to ensure thorough mixing. Break any bubbles that may have formed.
7. Read the absorbance on a microplate reader at a wavelength of 450 nm immediately. If wavelength correction is available, subtract readings at 570 nm from those at 450 nm to correct optical imperfections. Otherwise, read the plate at 450 nm only. Please note

that some unstable black particles may be generated at low concentration points after stopping the reaction for about 10 minutes, which will reduce the readings.

Download our ELISA guide for technical hints, results, calculation, and troubleshooting tips:

www.abcam.com/protocols/the-complete-elisa-guide

For all technical or commercial enquiries please go to:

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Additional information

CALCULATION

1. Calculate the mean value of the duplicate or triplicate readings for each standard and sample.
2. To generate a standard curve, plot the graph using the standard concentrations on the x-axis and the corresponding mean 450 nm absorbance (OD) on the y-axis. The best fit line can be determined by regression analysis using log-log or four-parameter logistic curve fit.
3. Determine the unknown sample concentration from the Standard Curve and multiply the value by the dilution factor.

TYPICAL DATA

Typical data provided for demonstration purposes only. A new standard curve must be generated for each assay performed.

Standard Point	ng/ml	OD	Average OD
1	16	0.260 0.242	0.251
2	4	0.410 0.386	0.398
3	1	0.795 0.841	0.818
4	0.25	1.335 1.389	1.362
5	0.063	1.768 1.828	1.798
6	0.00	2.055 2.127	2.091
Sample: Pooled Normal Sodium Citrate Human Plasma (1x)		1.354 1.378	1.366
Sample: Pooled Normal Human Serum (1x)		1.416 1.448	1.432

PERFORMANCE CHARACTERISTICS

1. The minimum detectable dose of DHT as calculated by 2SD from the mean of a zero standard was established to be 54 pg/ml.
2. Intra-assay precision was determined by testing three human plasma samples twenty times in one assay.
3. Inter-assay precision was determined by testing three human plasma samples in twenty assays.

	Intra-Assay Precision	Inter-Assay Precision
CV (%)	6.5	10.5

LINEARITY

Average Percentage of Expected Value (%)		
Sample Dilution	Human Plasma	Human Serum
1x	90	98
2x	107	102

CROSS REACTIVITY

Steroid	Cross-Reactivity (%)
DHT	100
Progesterone	None
Allopregnanolone	None
Cortexolone	None
Corticosterone	None
Deoxycorticosterone	None
Cortisone	None
6-Keto-17β-Estradiol	None
5-Androsten-3β-OL-7, 17-Dione	None
6-Keto-17α-Estradiol	None
3-Keto-5α, 16-Androstene	None
4-Androsten-17α-OL-3-One	None
Aldosterone	None
Ethynyl Estradiol	None
6-Ketoestriol	None
6-Ketoestrone	None
17β-Hydroxy-4-Androstene-3, 11-Dione	5
19-Nortestosterone	None
4-Pregnen-17, 20β-Diol-3-One	None
11α-Hydroxytestosterone	None
20α-Hydroxyprogesterone	None
6β-Hydroxyprogesterone	None
17-Hydroxyprogesterone	None
Cortisol	None
Testosterone	40

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