

# **DHT EXTRACTION**

**CAT # : ITN 9900.**

**Radioimmunoassay Kit for the quantitative  
measurement of DHT (DiHydroTestosterone) in  
Serum or plasma**

**PACK INSERT**

**I. Intended Use**

The IT DHT Extraction Radioimmunoassay Kit provides materials for the quantitative measurement of DHT in serum or plasma.

**II. Principle of the test**

The procedure follows the basic principle of radioimmunoassay whereby there is competition between a radioactive and a non-radioactive antigen for a fixed number of antibody binding sites (16). The amount of [I-125] labelled DHT bound to the antibody is inversely proportional to the concentration of DHT present in the serum. The separation of free and bound antigen is easily and rapidly achieved by using an accelerated double antibody Polyethylene glycol system.

**III. Reagents**

The DHT RIA Kit contains sufficient reagents for 100 determinations. Each kit contains the following reagents:

**A. DHT Standards:**

One vial of stock solution in alcohol, containing 100 ng/ml.

**Preparation of standard curve.**

Concentration (pg/ml)	Standard (ml)	Buffer (ml)	
2500	Stock : 0.1	3.9	= Sol A
1000	Stock : 0.1	9.9	= Sol B
500	Sol B : 1.0	1.0	= Sol C
200	Sol B : 0.2	0.8	= Sol D
100	Sol B : 0.1	0.9	= Sol E
50	Sol B : 0.1	1.9	= Sol F
25	Sol F : 0.5	0.5	= Sol G

The standard 0 pg/ml is the diluting buffer. Store at 2-8°C for up to 3 weeks.

**B. DHT I 125 Reagent : YELLOW**

One vial, 10.5 ml, containing 2µCi (74 kBq) of [I-125] labelled DHT in protein-based buffer containing 0.05% thimerosal as a preservative. Store at 2-8°C.

**C. Antibody Anti-DHT : BLUE**

Two vials, 5.2 ml, containing lyophilised anti-DHT rabbit serum (blue coloured) and 0.1% sodium azide as a preservative. Reconstitute by adding 5.2 ml of the blue coloured buffer. Store at 2-8°C.

**D. Precipitating Reagent: RED**

One vial, 105 ml, containing sheep anti-rabbit gamma globulin in a buffer, polyethylene glycol as a precipitating aid and 0.1% sodium azide as a preservative. Store at 2-8°C.

**E. Oxydation Solution**

One vial, 5 ml, containing KMnO<sub>4</sub> solution.

**F. Buffer (PBS)**

One vial, 100 ml.

All reagents and samples must be allowed to reach room temperature and mixed thoroughly by gentle inversion before use.

**IV. Precautions**

**For IN VITRO Diagnostic Use.**

**Radioactive Material - Not For Internal or External Use in Humans or Animals.**

**This radioactive material may be received, acquired, possessed, and used only by physicians, clinical laboratories or hospitals and only for IN VITRO clinical or laboratory tests not involving internal or external administration of the material, or the radiation therefrom to human beings or animals. Its receipt, acquisition, possession, use and transfer are subject to regulations.**

The following precautions should be observed in handling radioactive material:

1. Store radioactive materials in the original container, in a specifically designated, properly labelled area. Access to radioactive materials must be limited to authorised personnel only.
2. Do not eat, drink, smoke or apply cosmetics in areas where radioactive materials are stored or handled.
3. Do not pipette radioactive materials by mouth.
4. Always wear a protective laboratory coat and disposable gloves when handling radioactive materials. Wash hands thoroughly afterwards.
5. Cover working area with disposable absorbent paper.
6. Areas where spills occur should be wiped immediately with suitable absorbent material, which should then be disposed of as radioactive waste. The contaminated area should then be washed using an alkaline detergent or radiological decontamination solution.
7. Dispose of the radioactive waste into the sanitary sewage system if permitted by the local regulations.

**WARNING: POTENTIAL BIOHAZARDOUS MATERIAL**

This kit contains some reagents made with human serum or plasma. Because no test method can offer complete assurance that human T-Lymphotropic virus type III/Lymphadenopathy associated virus (HTLV-III/LAV), Hepatitis B virus, or other infectious agents are absent, these reagents should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control / National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories", 1984.

**V. Specimen collection and preparation**

Serum should be used and the usual precautions for venipuncture should be observed. The serum may be stored at 2-8°C for up to 24 hours and should be frozen at -15°C or lower for longer periods. Do not use grossly hemolysed or grossly lipemic specimens.

**VI. Procedural notes**

A thorough understanding of this package insert is necessary for successful use of the product. Reliable results will only be obtained by using precise laboratory techniques and accurately following the package insert. A standard curve must be included with each assay.

Do not mix various lots of any kit component within an individual assay. Do not use components beyond the expiration date shown on the kit label.

After removing assay reagents from the refrigerator, allow them to reach room temperature before pipeting. Unused reagents should be stored at 2-8°C. Standards and controls should be stored at -15°C if stored longer than 3 weeks. Standards and controls should be mixed before use by inverting or swirling gently rather than vortexing.

Failure to obtain the appropriate DHT values for controls may indicate imprecise manipulations, improper sample handling or deterioration of reagents. Inadequate decanting of tubes and/or failure to blot tubes following decantation may result in poor replication and spurious values.

**VII. Test procedure****A. Materials Supplied:**

Materials supplied in IT's DHT Radioimmunoassay Kit, Catalog No. ITN 9900:

Material	Quantity	Cat.No.
DHT Standard 100 ng/ml	one vial	9909
DHT Antiserum 5.2 ml	two vials	9910
DHT [I-125] Reagent	one vial	9920
Precipitating Reagent (Sheep Anti-Rabbit IgG/PEG)	one vial	0030
Oxydation Solution	one vial	9990
Buffer	one vial	9950

**B. Materials Required But Not Supplied**

1. Refrigerator (2-8°C).
2. 12 x 75 mm plastic or glass test tubes.
3. Test tube rack for 12 x 75 mm tubes.
4. Precision pipette to deliver 100 µl.
5. Precision repeating pipette to deliver 100 µl, 500 µl and 1 ml.
6. Controlled-temperature water bath, 37 ± 2°C.
7. Centrifuge (preferably refrigerated) capable of 1500 x g.
8. Absorbent material for blotting tubes.
9. Gamma counter.
10. Semi-log (linear-log) graph paper.
11. Vortex mixer.

**C. Preparation of the samples**

In glass tubes in duplicates:

Extract 300 µl of serum or plasma and controls by 3 ml of Diethyl Ether.

Vortex or shake for two minutes.

Centrifuge. Freeze aqueous layer in a freezer.

Transfer solvent into glass tubes and evaporate to dryness.

Add 0.5 ml of buffer and vortex for 30 seconds.

Add 50 µl of the oxydation solution.

Incubate at 37°C for 20 minutes.

Extract DHT by 3 ml of a mixture of Hexane/Ethanol 98/2. Vortex or shake for two minutes. Centrifuge.

Transfer 2 ml of the organic layer into glass tubes and evaporate to dryness.

The tubes are now ready for the RIA determination.

**D. Assay Procedure**

Allow all reagents to reach room temperature and mix liquid reagents thoroughly by gentle inversion before use. Standards, controls and unknowns should be assayed in duplicate.

The standard curve has to be prepared into glass tubes.

1. Label and arrange tubes in duplicate for total counts, non-specific binding, standards, controls and unknowns as shown in Table I.
2. Add 200 µl of the Buffer into extracted tubes and incubate for 20 minutes at room temperature. Vortex now and again.  
  
Add 200 µl of the prepared standards into glass tubes.
3. Add 100 µl of the antibody anti-DHT to all tubes except Total counts and NSB).
4. Add 100 µl of DHT [I-125] Reagent to each tube.
5. Vortex, cover and incubate all tubes at 37°C for 1 hour.
6. Add 1 ml of Precipitating Reagent to all tubes except the Total Count tubes and immediately vortex all tubes. THE PRECIPITATING REAGENT MUST BE MIXED THOROUGHLY BEFORE USE.
7. Incubate at room temperature for 10 to 15 minutes.
8. Centrifuge all tubes except the Total Count tubes for 15 minutes at 1500 x g.
9. Aspirate or decant and discard the supernatant by inverting the tubes. Blot the tubes to remove any droplets adhering to the rim before returning the tubes to the upright position.
10. Count the pellet remaining in the tubes in a gamma counter for one minute.
11. Construct a standard curve for each test run as described in Results.

**VIII. Results**

1. Calculate the average counts per minutes (CPM) for each standard, patient sample, and control. Subtract the average CPM of the NSB tubes from all counts to obtain corrected counts. Calculate the % bound or % B/Bo for each standard, patient sample, and control as follows:

$$\% \text{ Bound} = \frac{\text{Sample Counts} - \text{NSB Counts}}{\text{Average Total Counts}} \times 100$$

$$\% \text{ B/Bo} = \frac{\text{Sample Counts} - \text{NSB Counts}}{\text{Zero Standard Counts} - \text{NSB Counts}} \times 100$$

Plot a curve of radioactivity counts (CPM), % bound or % B/Bo for the DHT standards against the DHT concentration on a linear log graph paper. Draw a smooth curve through the mean of duplicate points. A typical set of data calculated in terms of % B/Bo is given in Table II

2. Determine the DHT concentration of the means of the duplicate counts of the unknown samples from the standard curve.
3. Any sample reading greater than 1 ng/ml should be diluted appropriately with 0 pg/ml standard and reassayed.

**IX. Limitations**

The reagents supplied in this kit are optimized to measure DHT levels in serum.

Repeated freezing and thawing of reagents supplied in the kit and of specimens must be avoided.

Hemolyzed and lipemic specimens may give false DHT values and hence must be avoided.

**X. Quality control**

1. Non-specific binding (NSB), or counts obtained in NSB tubes greater than 10%, may indicate reagent deterioration, contamination, or poor technique.
2. Maximum binding (% bound), or counts bound in the absence of unlabeled antigen, is approximately 50% when freshly iodinated tracer is used and may fall to 30% at the end of expiration date. Maximum binding less than 20% may indicate reagent deterioration, contamination, or poor technique.
3. IT controls or other commercial controls results should fall within the confidence limits established in each laboratory. The confidence limits for IT controls are printed on the Q.C. data sheet that accompanies each kit.

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**Table I: Outline of procedure**

Tube No.	Tube	Standards (μl)	Controls, unknown (μl of BUFFER)	DHT Anti-serum (μl)	DHT I-125 Reagent (μl)	1*	Precipitating Reagent (ml)	2*
1, 2	Total Counts	0		-	100		-	
3, 4	NSB	300*		-	100		1	
	DHT standards (pg/ml)							
5, 6	0	200	0	100	100		1	
7, 8	25	200	0	100	100		1	
9, 10	50	200	0	100	100		1	
11, 12	100	200	0	100	100		1	
13, 14	200	200	0	100	100		1	
15, 16	500	200	0	100	100		1	
17, 18	1000	200	0	100	100		1	
19, 20	2500	200	0	100	100		1	
	DHT serum controls							
21, 22	Level I	0	200	100	100		1	
23, 24	Level II	0	200	100	100		1	
25, 26	Unknown	0	200	100	100		1	
* Buffer NSB (Non-Specific Binding)								

(1\*). Vortex, Incubate at 37°C for 1 Hr.

(2\*). Vortex, Incubate at room temperature for 10 - 15 minutes.

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**Table II: Typical standard curve data**

Tube No.	Tube	Net average bound counts (cpm)	(%) B/Bo	DHT (ng/ml)
1, 2	Total Counts	36935		
3, 4	NSB	888	2.4	
	DHT standards (pg/ml)			
5, 6	0	27000	100.0	
7, 8	20	24747	91.4	
9, 10	50	23559	86.8	
11, 12	100	19816	72.5	
13, 14	200	15742	56.9	
15, 16	500	9844	34.3	
17, 18	1000	6277	20.6	
19, 20	2500	3319	9.3	
	DHT serum controls			
21, 22	Level I	4256	66	0.12
23, 24	Level II	3535	48	0.25
25, 26	Level III	2091	27	0.60

**CAUTION:** The above data must not be employed in lieu of data obtained by the user in the laboratory.

### XI. Expected Values

Normal ranges should be established by each laboratory using normal subjects. Results of normal range studies conducted on the ITN-9900 Kit by an independent laboratory are reported below:

		DHT (ng/ml)
Males	Pre-puberty	< 0.05
	Adult	0.25 - 1.00
Female	Follicular phase	0.05 - 0.20
	Luteal phase	0.10 - 0.30
	Menopausal	< 0.1

### XII. Performance characteristics

#### A. Sensitivity

The lowest detectable level of DHT that can be distinguished from the 0 pg/ml DHT Standard is  $\pm 4$  pg/tube ( $\pm 20$  pg/ml) at the 95% confidence limit.

#### B. Precision

Three samples were assayed to determine intra-assay and inter-assay precision.

	N	Mean (ng/ml)	Standard Deviation (ng/ml)	Coefficient of variation (%)
<b>Intra-Assay</b>				
Sample I	10	0.15	0.02	13.3
Sample II	10	0.40	0.03	7.5
Sample III	10	0.70	0.04	5.7
<b>Inter-Assay</b>				
Sample I	10	0.14	0.02	14.2
Sample II	10	0.41	0.04	9.7
Sample III	10	0.68	0.04	5.9

### C. Recovery

To assess the accuracy of the IT DHT RIA Kit, a recovery study was conducted. Two serum samples containing different levels of endogenous DHT were spiked with different amounts of a serum sample with an elevated level of DHT and assayed. Recovery is calculated as the percent of the observed divided by the expected concentration.

Sample	Endog. (ng/ml)	Spiked (ng/ml)	Expected (ng/ml)	Observ. (ng/ml)	Recov. (%)
I	0.12	0.10	0.22	0.20	91.0
		0.40	0.52	0.55	106.0
II	0.40	0.10	0.50	0.48	0.96
		0.40	0.80	0.76	0.95

The recovery found is from 75% to 85%.

### D. Parallelism Study

To further assess the accuracy of the IT DHT RIA Kit, a Parallelism study was conducted. A serum sample was diluted with the Free DHT Serum and assayed. Recovery is calculated as the percent of the observed divided by the expected concentration.

Dilution	Expected (ng/ml)	Observed (ng/ml)	Recovery (%)
-		0.8	
1:2	0.4	0.42	105
1:4	0.2	0.19	95
1:8	0.1	0.095	95
1:16	0.05	0.045	90

### E. Specificity

The Cross-reactivity of the DHT antiserum has been measured against various compounds. The percent cross-reactivity is expressed as the ratio

of the DHT concentration to the concentration of the reacting compound at 50% binding of the 0 pg/ml Standard.

Steroid	% Cross-reactivity
DHT	100.00
Estradiol	1.20
Estriol	0.04
DES	< 0.01
Pregnenolone	<0.01
Progesterone	<0.01
Testosterone	<0.01
Aldosterone	<0.01

**XIII. References**

1. Yalow, R. and Berson, S. : Principles of Competitive Protein Binding Assays. Odell, W. and Daughaday, W. (eds.), Ch. 1: 1971. J.B. Lippincott Co., Philadelphia.