BioVendor Group

ENG

Instructions for Use:

DIHYDROTESTOSTERONE (DHT) ELISA

Catalogue number:

RCD009R

For research use only.

BioVendor R&D

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CONTENT

HISTORY OF CHANGES

Previous version	Current version
ENG.005.A	ENG.006.A
Editing the format, changing the structure of the document chapters	
Chapter 1 : Added: *This kit is intended for professional use only and is for laboratory use only. For in vitro use only. Intended to be used manually but may be adaptable to pen automated analyzers. The user is responsible for validating the performance if this kit with automated analyzers*	
Chapter 2. added	
Chapter 5. updated	
Chapter 8. updated	
Chapter 9. updated	
Chapter 12.3. updated	
Chapter 13, point 7 updated	
Chapter 14, point 3,4, updated	
Chapter 15 added	
Chapter 18.3 updated	
Chapter 18.4.2. new data added	

1. INTENDED USE

For the quantitative determination of dihydrotestosterone (DHT) in human serum by an enzyme immunoassay.
This kit is intended for professional use only and is for laboratory use only. For *in vitro* use only. Intended to be used manually but may be adaptable to pen automated analyzers. The user is responsible for validating the performance if this kit with automated analyzers.

2. LIMITATIONS

- This test is not intended to be used for screening purposes
- This test is not intended for home testing or self-testing
- This kit is calibrated for the determination of DHT in human serum. This kit is not calibrated for the determination of DHT in other specimens of human or animal origin
- The results obtained with this kit shall never be used as the sole basis for clinical diagnosis and for therapeutic decisions
- Although common interfering substances have been evaluated with this test, other substances that have not been evaluated such as drugs and the occurrence of heterophilic antibodies in individuals regularly exposed to animals or animal products have the potential of causing interferences

3. SUPPLEMENTAL INFORMATION

Dihydrotestosterone (DHT) is the most active natural androgen in humans with a principal role in the development of primary and secondary sexual characteristics and potential participation in a myriad of other physiological processes. The bulk of androgen production takes place mainly in the Leydig cells of the testes. Androgens circulate in the blood bound to proteins, especially sex hormone binding globulin (SHBG) from peripheral conversion of testosterone, while in females most of the DHT is derived from androstenedione.
Some of the main clinical indications of the DHT measurement in serum are investigations of delayed puberty in men and evaluation of the presence of active testicular tissue[1].

4. PRINCIPLE OF THE TEST

The DHT ELISA is a competitive immunoassay. Competition occurs between DHT present in calibrators, controls, specimen samples and an enzyme-labelled antigen (HRP conjugate) for a limited number of anti-DHT antibody binding sites on the microplate wells. After a washing step that removes unbound materials, the TMB substrate (enzyme substrate) is added, which reacts with HRP to form a blue-coloured product that is inversely proportional to the amount of DHT present. Following an incubation, the enzymatic reaction is terminated by addition of stopping solution. The absorbance is measured with a microplate reader at 450 nm. A set of calibrators is used to plot a calibrator curve from which the amount of DHT in specimen samples and controls can be directly read.

5. PROCEDURAL CAUTIONS AND WARNINGS

1. This kit is for professional use only and for in vitro use only.
2. Practice good laboratory practices when handling kit reagents and specimens. This includes:

– Do not pipette by mouth.
– Do not smoke, drink, or eat in areas where specimens or kit reagents are handled.
– Wear protective clothing and disposable gloves.
– Wash hands thoroughly after performing the test.
– Avoid contact with eyes; use safety glasses; in case of contact with eyes, flush eyes with water immediately and contact a doctor.

3. Users should have a thorough understanding of this protocol for the successful use of this kit. Reliable performance will only be attained by strict and careful adherence to the instructions provided.
4. Do not use the kit beyond the expiry date stated on the label.
5. If the kit reagents are visibly damaged do not use the test kit.
6. Do not use kit components from different kit lots within a test and do not use any component beyond the expiration date printed on the label.
7. All kit reagents and specimens must be brought to room temperature and mixed gently but thoroughly before use. Avoid repeated freezing and thawing of specimens.
8. When the use of water is specified for dilution or reconstitution, use deionized or distilled water.
9. Immediately after use, each individual component of the kit must be returned to the recommended storage temperature stated on the label.
10. A calibrator curve must be established for every run.

11. It is recommended to all customers to prepare their own control materials or serum pools which should be included in every run at a high and low level for assessing the reliability of results.
12. The controls (included in kit) must be included in every run and their results must fall within the ranges stated in the quality control certificate; a failed control result might indicate improper procedural techniques or pipetting, incomplete washing or improper reagent storage.
13. When dispensing the substrate and stopping solutions, do not use pipettes in which these liquids will come into contact with any metal parts.
14. The TMB substrate is sensitive to light and should remain colorless if properly stored. Instability or contamination may be indicated by the development of a blue colour, in which case it should not be used.
15. Do not use grossly hemolyzed, grossly lipemic, icteric or improperly stored serum.
16. Samples or controls containing azide or thimerosal are not compatible with this kit, they may lead to false results.
17. Samples values above the measuring range of the kit may be reported as > 2500 pg/mL. If further dilution and retesting is required, only serum samples with a known low DHT concentration (< 50 pg/mL) may be used to dilute serum samples. The use of any other reagent will lead to false results.
18. Avoid microbial contamination of reagents.
19. To prevent contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, calibrator and control.
20. To prevent contamination of reagents, do not pour reagents back into the original containers
21. Kit reagents must be regarded as hazardous waste and disposed of according to local and/or national regulations.
22. Consumables used with the kit that are potentially biohazardous (e.g. pipette tips, bottles or containers containing human materials) must be handled according to biosafety practices to minimize the risk of infection and disposed of according to local and/or national regulations relating to biohazardous waste.
23. This kit contains 1 M sulfuric acid in the stopping solution component. Do not combine acid with waste containing sodium azide or sodium hypochlorite.
24. The use of safety glasses, and disposable plastic, is strongly recommended when manipulating biohazardous or bio-contaminated solutions.
25. Proper calibration of the equipment used with the test, such as the pipettes and absorbance microplate reader, is required.
26. If microplate shaker is required for the assay procedure, the type and speed of shaker required is stated in chapter 11. Both the type and speed of shaker used can influence the optical densities and test results. If a different type of shaker and/or speed is used, the user is responsible for validating the performance of the kit.
27. Do not reuse the microplate wells, they are for single use only.
28. To avoid condensation within the microplate wells in humid environments, do not open the pouch containing the microplate until it has reached room temperature.
29. When reading the microplate, the presence of bubbles in the wells will affect the optical densities (ODs). Carefully remove any bubbles before performing the reading step.
30. Any serious incident that has occurred in relation to the device shall be reported to the manufacturer.

6. LIMITATIONS

1. The kit is calibrated for the direct determination of DHT in human serum. The kit is not calibrated for the determination of DHT in other specimens of human or animal origin.
2. The results obtained with this kit shall never be used as the sole basis for a clinical diagnosis. For example, some drugs and the occurrence of heterophilic antibodies in patients regularly exposed to animals or animal products have the potential of causing interferences in immunological tests. Consequently, the clinical diagnosis should comprise all aspects of a patient's background including the frequency of exposure to animals/products.

7. SAFETY CAUTIONS AND WARNINGS

The reagents should be considered a potential biohazard and handled with the same precautions applied to blood specimens. All human specimens should be considered a potential biohazard and handled as if capable of transmitting infections and in accordance with good laboratory practices. The calibrators and controls provided with the kit contain processed human plasma that has been tested by approved methods and found to be negative for the presence of HBsAg and antibodies to HCV, HIV 1/2 and HIV NAT. However, no test method can offer complete assurance that any viable pathogens are absent. Therefore, these components should be considered a potential biohazard and handled with the same precautions as applied to any blood specimen, following good laboratory practices.

8. CHEMICAL HAZARDS

Avoid direct contact with any of the kit reagents. Specifically avoid contact with the TMB Substrate (contains tetramethylbenzidine) and Stopping solution (contains sulfuric acid). If contacted with any of these reagents, wash with plenty of water and refer to SDS for additional information.

9. SPECIMEN COLLECTION AND STORAGE

Approximately 0.1 mL of serum is required per duplicate determination. Collect 4–5 mL of venous blood into an appropriately labelled tube and allow it to clot. Centrifuge at room temperature and carefully transfer the serum into a new storage or container. Serum samples may be stored at room temperature for up to seven days, at 2–8°C for up to fourteen days or freeze at or below -20°C for up to 1 month.
Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

10. SPECIMEN PRE-TREATMENT

No specimen pre-treatment is necessary.

11. REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED

1. Calibrated single-channel pipette to dispense 50 µL.
2. Calibrated multi-channel pipettes to dispense 50 µL, 100 µL and 150 µL.
3. Calibrated multi-channel pipette to dispense 350 µL (for manual washing only).
4. Automatic microplate washer (recommended).
5. Disposable pipette tips.
6. Distilled or deionized water.
7. Absorbance microplate reader with a 450 nm filter and an upper OD limit of 3.0 or greater.

12. REAGENTS PROVIDED

12.1 Microplate

Ready To Use
Contents: One 96-well (12x8) polyclonal antibody-coated microplate in a resealable pouch with desiccant.
Storage: 2–8°C
Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks.

12.2 HRP Conjugate

Ready To Use
Contents: One bottle containing DHT-HRP conjugate in a protein-based buffer with a non-mercury preservative.
Volume: 15 mL/bottle
Storage: 2–8°C
Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks.

12.3 Calibrators A-G

Ready To Use
Contents: Seven bottles of calibrator containing specified DHT concentrations. Human serum-based matrix with a non-mercury preservative. Prepared by spiking matrix with defined quantities of DHT.
Listed below are approximate concentrations, please refer to QC Certificate label for exact concentrations.
Concentrations: 0, 25, 100, 250, 500, 1000, 2500 pg/mL
Volume: 1.0 mL/bottle
Storage: 2–8°C
Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks.

12.4 Controls

Ready To Use
Contents: Two bottles of control containing different DHT concentrations. Human serum-based matrix with a non-mercury preservative. Prepared by spiking matrix with defined quantities of DHT.
Refer to QC certificate for the acceptable ranges.
Volume: 1.0 mL/vial
Storage: 2–8°C
Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks.

12.5 TMB Substrate

Ready To Use
Contents: One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
Volume: 16 mL/bottle
Storage: 2–8°C

Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks.

12.6 Stopping Solution

Ready To Use
Contents: One bottle containing 1M sulfuric acid.
Volume: 8 mL/bottle
Storage: 2–8°C
Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks.
Safety: Refer to product SDS

12.7 Wash Buffer Concentrate

Concentrated; Requires Preparation
Contents: One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
Volume: 50 mL/bottle
Storage: 2–8°C
Stability: Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks at 2–8°C. Following Preparation: The wash buffer working solution is stable for 2 weeks following preparation, assuming Good Laboratory Practices are adhered to. To prevent microbial growth, prepare the wash buffer working solution in a clean container and store under refrigerated conditions (2-8°C) when not in use
Preparation of Wash Buffer Working Solution: Dilute 1:10 in distilled or deionized water before use. If the whole microplate is to be used, dilute 50 mL of the wash buffer concentrate to 450 mL of distilled or deionized water.

13. ASSAY PROCEDURE

All kit components, controls and specimen samples must reach room temperature prior to use. Calibrators, controls and specimen samples should be assayed in duplicate. Once the procedure has been started, all steps should be completed without interruption.

1. After all kit components have reached room temperature, mix gently by inversion.

2. Prepare the Wash Buffer Working Solution (See the *Reagents Provided* section, *Wash Buffer Concentrate*).

3. Plan the microplate wells to be used for calibrators, controls and samples. *See Recommended Assay Layout* section.
   Remove the strips that will not be used from the microplate frame and place them in the bag with desiccant. Reseal the bag with the unused strips and return it to the refrigerator.

4. Pipette **50 µL** of each calibrator, control, and specimen sample into assigned wells.

5. Pipette **100 µL** of the HRP conjugate into each well (the use of a multi-channel pipette is recommended).

6. Gently tap the microplate frame for 10 seconds to mix the contents of the wells and **incubate** the microplate at room temperature (no shaking) for **90 minutes**.

7. Wash the wells with an automatic microplate washer (preferred) or manually as stated below.

Automatic: Using an automatic microplate washer, perform a 3-cycle wash using **350 µL/well** of Wash Buffer Working Solution (3x350 µL). One cycle consists of aspirating all wells then filling each well with 350 µL of Wash Buffer Working Solution. After the final wash cycle, aspirate all wells and then tap the microplate firmly against absorbent paper to remove any residual liquid.

Manually:
For manual washing, perform a 3-cycle wash using **350 µL/well** of Wash Buffer Working Solution (3x350 µL). One cycle consists of aspirating all wells by briskly emptying the contents of the wells over waste container, then pipetting 350 µL of Wash Buffer Working Solution into each well using a multi-channel pipette. After the final wash cycle, aspirate all wells by briskly emptying the contents over a waste container and then tap the microplate firmly against absorbent paper to remove any residual liquid.

8. Pipette **150 µL** of TMB Substrate into each well (the use of a multi-channel pipette is recommended).

9. Incubate the microplate at room temperature (no shaking) for **30 minutes**.

10. Pipette **50 µL** of Stopping Solution into each well (the use of a multi-channel pipette is recommended) in the same order and speed as was used for addition of the TMB Substrate. Gently tap the microplate frame to mix the contents of the wells.

11. Measure the optical density (absorbance) in the microplate wells using an absorbance microplate reader set to 450 nm, within 20 minutes after addition of the stopping solution.

14. CALCULATIONS

1. Calculate the mean optical density of each calibrator, control and specimen sample.
2. Use a 4-parameter or 5-parameter curve fit with immunoassay software to generate a calibrator curve.
3. The immunoassay software will calculate the concentrations of the controls and specimen samples using the mean optical density values and the calibrator curve.
4. If a sample reads more than 2500 pg/mL and needs to be diluted and retested, then dilute with a serum sample with a known low DHT concentration (< 50 pg/mL) not more than 1:10. The result obtained must be multiplied by the dilution factor.

15. QUALITY CONTROL

When assessing the validity of the test results, the following criteria should be evaluated:

1. The calibrator A mean optical density meets the acceptable range as stated in the QC Certificate.
2. The calibrator with the highest concentration meets the % binding acceptable range as stated in the QC certificate. % Binding = (OD of calibrator/OD of calibrator A) x 100.
3. The values obtained for the kit controls are within the acceptable ranges as stated in the QC certificate.
4. The results of any external controls that were used meet the acceptable ranges.

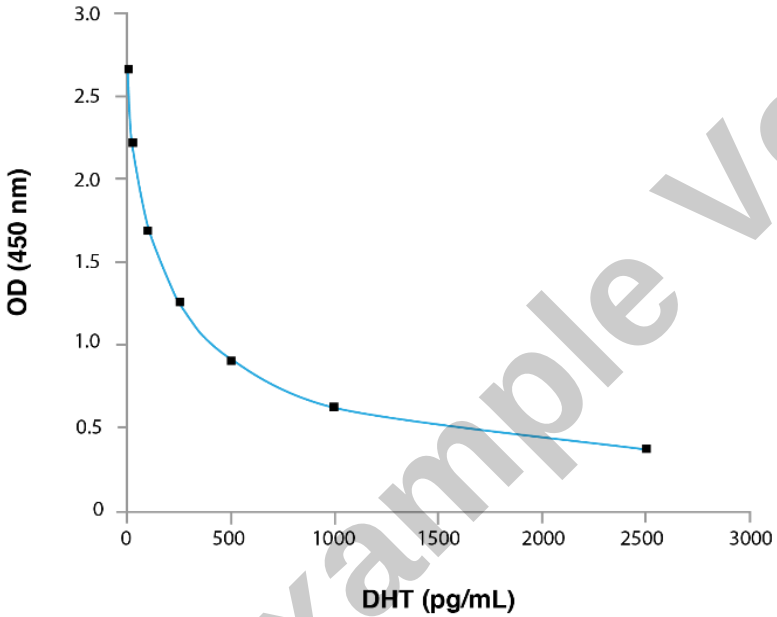
16. TYPICAL TABULATED DATA

Sample data only. Do not use to calculate results.

Calibrator	Mean OD (450 nm)	% Binding	Value (pg/mL)
A	2.664	100	0
B	2.225	84	25
C	1.695	64	100
D	1.261	47	250
E	0.911	34	500
F	0.622	23	1000
G	0.372	14	2500
Unknown	1.077	-	353

17. TYPICAL CALIBRATOR CURVE

Sample curve only. Do not use to calculate results.



18. PERFORMANCE CHARACTERISTICS

18.1 SENSITIVITY

The analytical sensitivity study was performed according to the CLSI EP17-A2 guideline. Sixty replicates of the matrix and low concentration samples were run in independent tests with three lots of the kit. The Limit of Background (LoB) was determined to be 9.4 pg/mL, the Limit of Detection (LoD) was determined to be 17.0 pg/mL and the Limit of Quantitation (LoQ) was determined to be 17.0 pg/mL.

18.2 SPECIFICITY (CROSS-REACTIVITY)

The following compounds were tested for cross-reactivity with DHT cross-reacting at 100%.

Compound	% Cross-Reactivity
5α-DHT	100
17-hydroxyprogesterone	< 0.01
17β-estradiol	< 0.01
Aldosterone	< 0.01
Androstenedione	0.6
Corticosterone	< 0.01
Cortisol	< 0.01
Danazol	< 0.01
DHEAS	< 0.01
Estriol	< 0.01
Estrone	< 0.01
Ethisterone	0.03
Pregnenolone	< 0.01
Progesterone	<0.01
Testosterone	8.1

18.3 INTERFERENCES

An interference study was performed according to the CLSI EP07-A2 guideline. No significant interference was observed for concentrations of up to 10 g/L Haemoglobin, 10 mg/dL Bilirubin (conjugated and unconjugated), 1500 mg/dL Triglycerides, 2.4 µg/mL Biotin, 1.2 µg/mL HAMAS, and 2531 IU/mL Rheumatoid Factor
Interferences were observed for both bilirubin conjugated and unconjugated at levels of 20 mg/dL or higher.

18.4 PRECISION

A precision study was performed according to the CLSI EP05-A2 guideline.

18.4.1 Repeatability

The experimental protocol used a nested components-of-variance design with 7 serum samples, 10 testing days, two lots and two scientists per day. Each scientist ran two tests per day and two replicate measurements per run (a 10 x 2 x 2 x 2 design) for each sample. The results were analyzed with a two-way nested ANOVA and summarized in the table below.

Sample	Mean, (pg/mL)	Within Run SD (pg/mL)	Within Run CV%	Between Run SD (pg/mL)	Between Run CV%	Total SD (pg/mL)	Total CV%
1	31.4	13.7	43.7	3.3	10.5	14.1	44.9*
2	144.2	19.3	13.4	8.5	5.9	21.0	14.6
3	817.5	51.7	6.3	21.1	2.6	55.8	6.8
4	429.5	34.5	8.0	10.8	2.5	36.8	8.6
5	586.2	38.8	6.6	15.5	2.6	41.8	7.1
6	1561	90.0	5.8	24.1	1.5	94.5	6.1
7	1287	71.1	5.5	18.5	1.4	73.4	5.7

* Samples that are close to the limit of quantitation are expected to have a higher imprecision. The allowable total error for samples lower than 145 pg/mL is ± 30 pg/mL.[6]

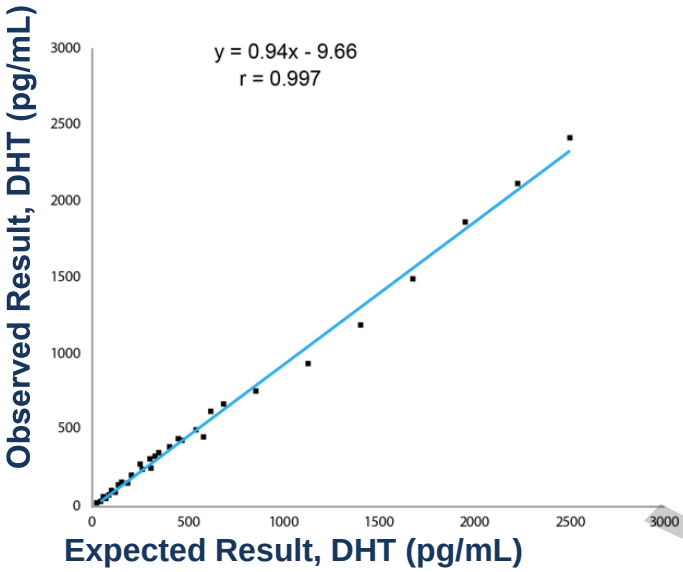
18.4.2 Reproducibility

The reproducibility study evaluated the precision performance of the device following experimental design model 3 x 5 x 5 (3 locations x five testing days x five replicates per day) across laboratories. The results were analyzed with two-way nested ANOVA and are summarized in the table below.

Sample	Mean (mg/mL)	Repeatability		Within Location		Reproducibility	
		SD (pg/mL)	CV%	SD (pg/mL)	CV%	SD (pg/mL)	CV%
QCL	129.4	5.5	4.3	6.5	5.1	7.5	5.8
QCH	411.8	14.5	3.5	18.4	4.5	19.8	4.8
1	65.4	4.4	6.7	5.7	8.8	10.7	16.4
2	189.7	8.5	4.5	17.7	9.3	33.5	17.7
3	228.2	8.8	3.9	15.7	6.9	30.6	13.4
4	390.4	12.1	3.1	31.1	8.0	38.0	9.7
5	655.8	18.7	2.8	38.3	5.8	63.8	9.7
6	883.2	26.4	3.0	55.4	6.3	128.1	14.5

18.5 LINEARITY

The linearity study was performed according to the CLSI guideline EP06-Ed2 guideline using four human serum samples covering the range of the assay (between 226 and 2500 pg/mL).
The samples were diluted in serum samples with a low concentration of DHT (less than 50 pg/mL) at several equidistant concentration levels and up to ten percent (1:10), tested in duplicate, and the results compared to the predicted concentration. The statistical analysis shows that the assay is sufficiently linear up to a 1:10 dilution.



18.6 RECOVERY

Spiked samples were prepared by adding defined amounts of DHT (present in serum samples with a high DHT concentration) to four serum samples. The results are tabulated below:

Sample	Concentration Result (pg/mL)	Concentration of Spiking Samples (pg/mL)	Expected Concentration from 9:1 v/v (pg/mL)	Recovery %
1	**91.3**	-	-	-
	177.3	800	162.2	109.3
	230.2	1472	229.4	100.3
	313.8	2672	349.4	89.8
2	**191.6**	-	-	-
	261.0	800	252.5	103.4
	306.2	1472	319.7	95.8
	408.0	2672	439.7	92.8
3	**379.5**	-	-	-
	433.2	800	421.6	102.7
	499.5	1472	488.8	102.2
	573.3	2672	608.8	94.2
4	**360.2**	-	-	-
	383.2	800	404.2	94.8
	461.8	1472	471.4	98.0
	510.8	2672	591.4	86.4

18.7 COMPARATIVE STUDIES

The DHT ELISA kit (y) was compared to a Liquid Chromatography-Tandem Mass Spectrometry DHT method (x). The comparison of 90 serum samples yielded the following linear regression results using a Passing-Bablok fit: y = 0.78x + 73.8, r = 0.88.

18.8 REFERENCE RANGES

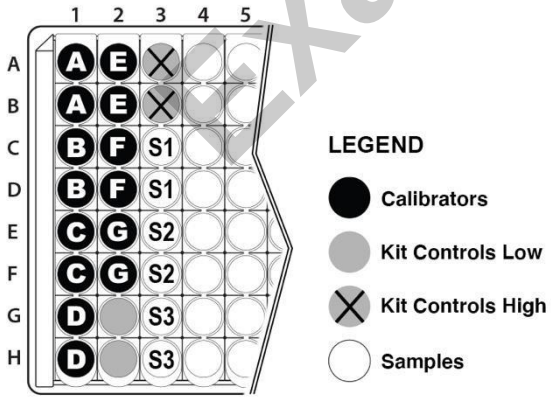
Reference ranges (95%) were estimated using samples obtained from adult individuals of diverse races. Each laboratory shall establish their own range of reference values. ND = Not detectable; lower than the LoD.

Cohort	N	Median (pg/mL)	95% Reference Range (pg/mL)
Adult Males (20–89 years old)	304	380	143 – 842
Adult Females (18–50 years old)	183	91	ND – 596
Adult Females (51–83 years old)	135	53	ND – 431

Reference ranges were estimated using pediatric samples as shown below. Due to the limited sample size, a 95% reference range could not be established; the total range is provided. Each laboratory shall establish their own range of reference values.

Gender	Age (years)	N	Total Range (pg/mL)
Male	1–9	40	ND – 85.7
	10–14	26	11.1 – 875.6
	15–18	14	70.3 – 1260.9
Female	2–9	40	ND – 88.9
	10–14	21	22.5 – 280.6
	15–18	19	62.6 – 760.3

19. RECOMMENDED ASSAY LAYOUT



20. REFERENCES

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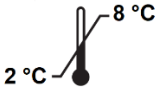
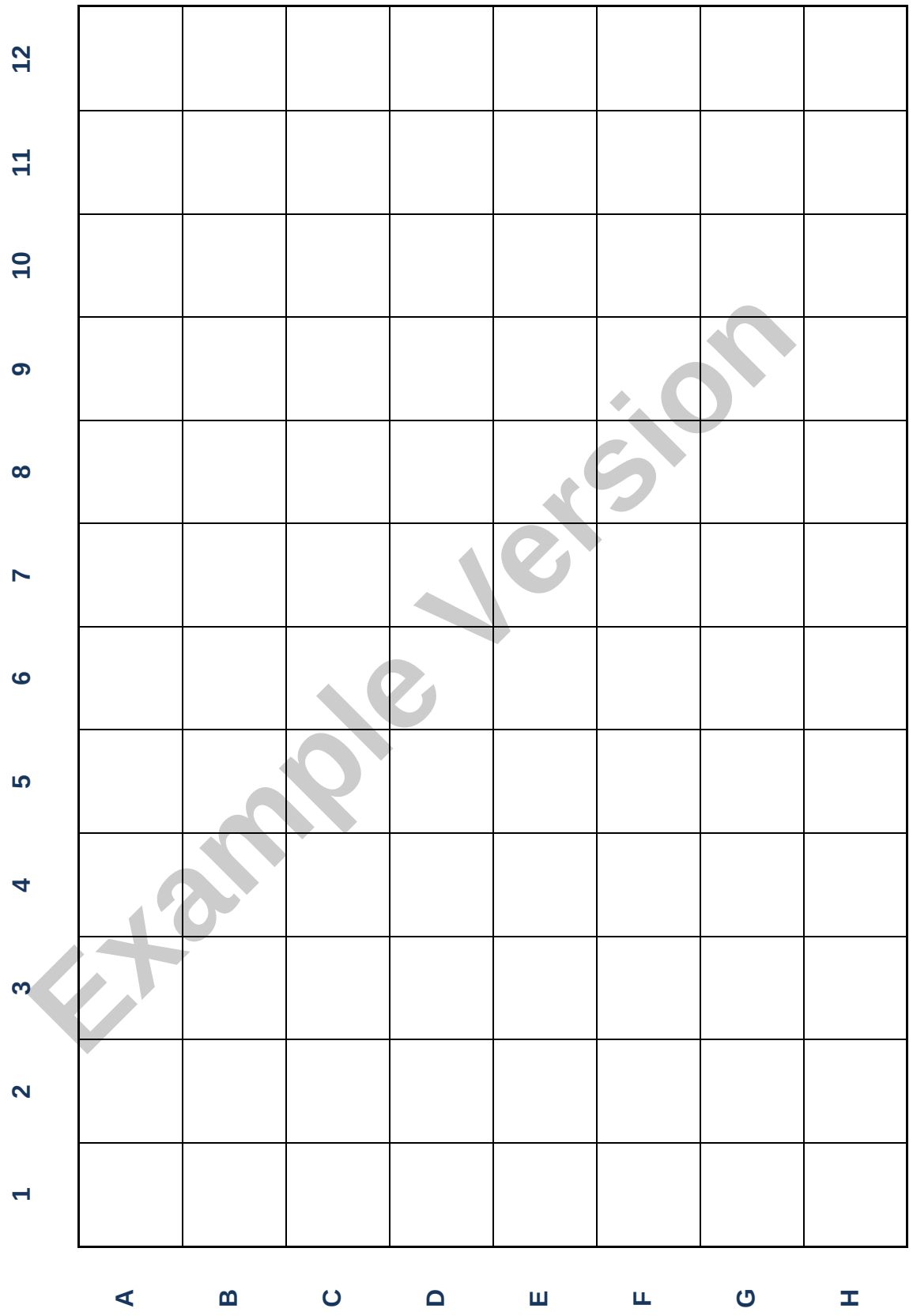
21. EXPLANATION OF SYMBOLS

	Catalogue number
	Batch code
	Caution
	Use by date
	Temperature limit
	Manufacturer
	Read electronic instructions for use - eIFU
	The content is sufficient for 96 tests
	Biological risks



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