BioVendor Group

ENG

Instructions for Use:

HUMAN TRIIODOTHYRONINE TOTAL (T3) ELISA

Catalogue number:

RCD025R

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
Chapter 5. : point 30 added	
Chapter 9: point 7. Wash Buffer concentrate: added details on stability	

1. INTENDED USE

For the quantitative measurement of Triiodothyronine (T3) in human serum by an ELISA (Enzyme-Linked Immunosorbent Assay). Results shall be used in combination with other clinical and laboratory data to aid in the diagnosis of thyroid diseases in the general population.
This kit is intended for professional use only and is for laboratory use only. For *research* use only. Intended to be used manually but may be adaptable to open automated analyzers. The user is responsible for validating the performance of this kit with any automated analyzers.

2. LIMITATIONS RELATED TO INTENDED PURPOSE & USE

1. This test is not intended to be used for screening purposes.
2. This test is not intended for home testing or self-testing.
3. The kit is calibrated for the determination of triiodothyronine in human serum. The kit is not calibrated for the determination of triiodothyronine in other specimens of human or animal origin.
4. The results obtained with this kit shall never be used as the sole basis for a clinical diagnosis and for therapeutic decisions.
5. 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

Triiodothyronine (T3) and thyroxine (T4) are the two active thyroid hormones found in the blood stream. About 80% of T3 is produced by the deiodination of T4 in the peripheral tissue and the other 20% is produced directly from the thyroid gland. T3 is transported through the peripheral blood stream bound to serum proteins, namely thyroxine binding globulin, thyroid binding prealbumin and albumin. About 0.3% of the total T3 is unbound and is therefore considered the free fraction. T3 has an influence on oxygen consumption and heat production in virtually all tissues. The hormone also plays a critical role in growth, development, and sexual maturation of growing organisms.
T3 is one parameter used in the clinical diagnosis and differentiation of thyroid disease, particularly hyperthyroidism. In most hyperthyroid patients, both serum T3 and serum T4 levels are elevated. Serum T3 levels are a sensitive indicator of the impending hyperthyroid state often preceding elevated T4 and free thyroxine index values. Serum T3 levels are clinically significant in both the diagnosis of thyroid disease and in the detection of T3-thyrotoxicosis. However, it has been demonstrated that T3 levels may be affected by a number of medications, acute and chronic stress, and a variety of acute and chronic non-thyroidal illnesses. It is therefore necessary to differentiate those results that are due to thyroid dysfunction from those related to non-thyroidal diseases.

4. TEST PRINCIPLE

The T3 ELISA is a competitive immunoassay. Competition occurs between T3 present in calibrators, controls, specimen samples and an enzyme-labelled antigen (HRP conjugate) for a limited number of anti-T3 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 T3 present. Following an incubation, the enzymatic reaction is terminated by the addition of the stopping solution, converting the blue colour to a yellow colour. The absorbance is measured on a microplate reader at 450 nm. A set of calibrators is used to plot a calibrator curve from which the amount of T3 in specimen samples and controls can be directly read.

5. PROCEDURAL CAUTIONS AND WARNINGS

1. This kit is for use by trained laboratory personnel (professional use only). For laboratory 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 colourless 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 >10 ng/mL. If further dilution and retesting is required, only calibrator A may be used to dilute serum samples. The use of any other reagent may lead to false results.
18. Avoid microbial contamination of reagents.
19. To prevent the contamination of reagents, use a new disposable pipette tip for dispensing each reagent, sample, calibrator, and control.
20. To prevent the 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 material 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 a microplate shaker is required for the assay procedure, the type and speed of shaker required is stated in the REAGENTS AND EQUIPMENT NEEDED BUT NOT PROVIDED section. 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. Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.
30. 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.

6. SAFETY CAUTIONS AND WARNINGS

6.1 Biohazards

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 serum/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.

6.2 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.

7. SPECIMEN COLLECTION, STORAGE AND PRE-TREATMENT

7.1 Specimen Collection & Storage

Approximately 0.15 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 tube or container.
Serum samples may be stored at 2-8°C for up to 3 days or at -20°C or lower for up to 3 months. Specimens may be more stable than indicated.
Consider all human specimens as possible biohazardous materials and take appropriate precautions when handling.

7.2 Specimen Pre-Treatment

Specimen pre-treatment is not required.

8. 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 pipettes to dispense 350 µL (if washing manually).
4. Automatic microplate washer (recommended).
5. Disposable pipette tips.
6. Distilled or deionized water.
7. Calibrated absorbance microplate reader

9. REAGENTS PROVIDED

1.Microplate

Contents:	One anti-T3 polyclonal antibody-coated 96-well (12x8) microplate in a resealable pouch with desiccant.
Format:	Ready to Use
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks

2. HRP Conjugate

Contents:	One bottle containing T3-Horse Radish Peroxidase (HRP) conjugate in a protein-based buffer with a non-mercury preservative.
Format:	Ready to Use
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.

3. Calibrator A-E

Contents:	Five bottles of calibrator containing specified T3 concentrations. Human serum-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of T3. Listed below are approximate concentrations, please refer to vial labels for exact concentrations. Concentrations: 0, 0.3, 1, 3, 10 ng/mL.
Format:	Ready to Use
Volume:	Calibrator A: 2.0 mL/bottle Calibrator B-E: 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.

4. Control 1-2

Contents:	Two bottles of control containing different T3 concentrations. Human serum-based buffer with a non-mercury preservative. Prepared by spiking buffer with defined quantities of T3. Refer to the QC certificate for the target values and acceptable ranges.
Format:	Ready to Use
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.

5. TMB Substrate

Contents:	One bottle containing tetramethylbenzidine and hydrogen peroxide in a non-DMF or DMSO containing buffer.
Format:	Ready to Use
Volume:	18 mL/bottle
Storage:	2–8°C
Stability:	Unopened: Stable until the expiry date printed on the label. After Opening: Stable for four weeks.

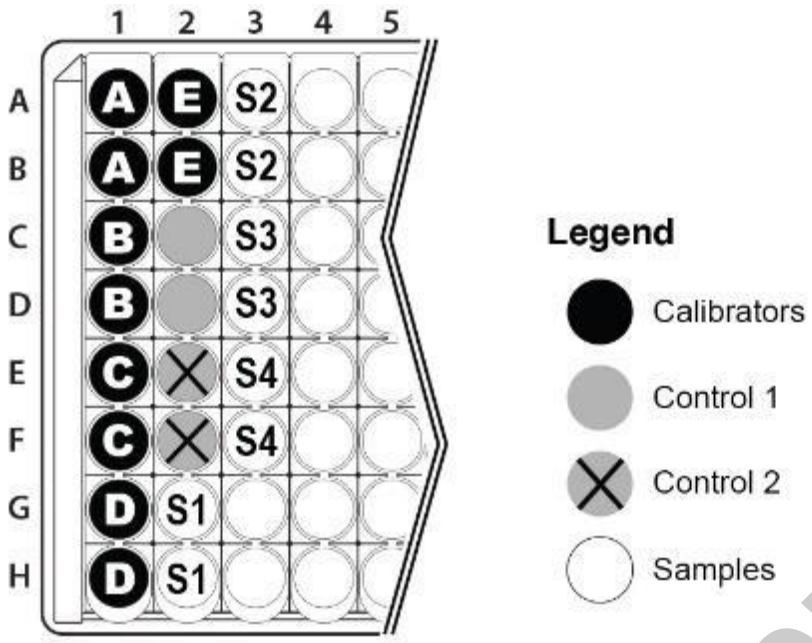
6. Stopping Solution

Contents:	One bottle containing 1M sulfuric acid.
Format:	Ready to Use
Volume:	6 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.

7. Wash Buffer concentrate

Contents:	One bottle containing buffer with a non-ionic detergent and a non-mercury preservative.
Format:	Concentrated; Requires Preparation
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. 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:	**X10 Dilute 1:10 Before Use** 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 in 450 mL of distilled or deionized water.

10. RECOMMENDED ASSAY LAYOUT



11. PREPARATION OF REAGENTS

Specimen Pre-Treatment: None

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 section *9. Reagents Provided, 7. Wash Buffer Concentrate*).
3.	**Plan** the microplate wells to be used for calibrators, controls, and samples. See section *10. Recommended Assay Layout.* Remove the strips from the microplate frame that will not be used 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 **60 minutes**.
7.	**Wash** the microplate 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 (3 x 350 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 (3 x 350 µL). One cycle consists of aspirating all wells by briskly emptying the contents of the wells over a waster 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 15 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.

12. CALCULATIONS

1. Calculate the mean optical density for each calibrator, control and specimen sample duplicate.
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 10 ng/mL and needs to be diluted and retested, then dilute with calibrator A not more than 1:5. The result obtained must be multiplied by the dilution factor

13. 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.

14. TYPICAL DATA

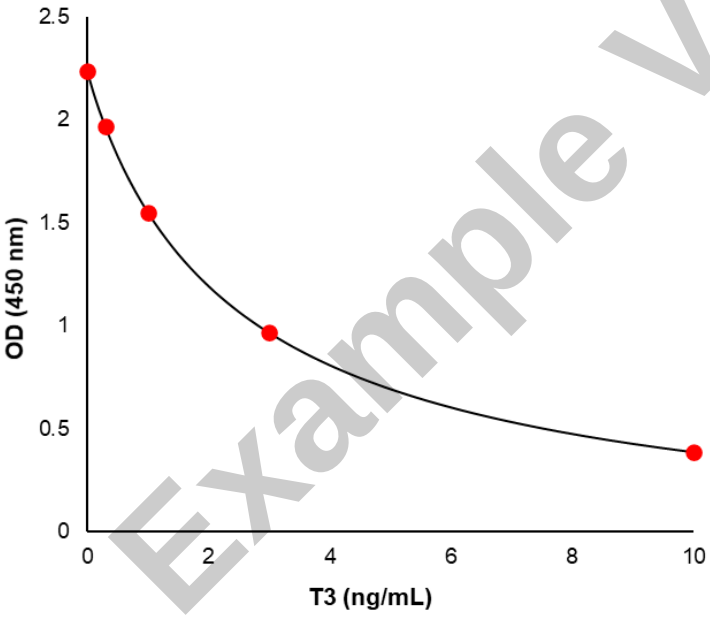
14.1 Typical tabulated data

Sample data only. **Do not** use to calculate results

. Calibrator	Mean OD (450 nm)	% Binding	Value (ng/mL)
A	2.239	100	0
B	1.969	88	0.3
C	1.549	69	1
D	0.966	43	3
E	0.383	17	10
Unknown1	1.512	-	1.1

14.2 Typical calibrator curve

Sample curve only. **Do not** use to calculate results.



15. PERFORMANCE CHARACTERISTICS

15.1 Sensitivity

The analytical sensitivity study was performed according to the CLSI EP17-A2 guideline. The Limit of Background (LoB), Limit of Detection (LoD) and Limit of Quantitation (LoQ) are summarized in the table below:

Parameter	T3 (ng/mL)
LoB	0.082
LoD	0.153
LoQ	0.280

15.2 Specifity (Cross-reactivity)

The following compounds were tested for cross-reactivity with T3 cross-reacting at 100%.

Compound	% Cross-Reactivity
3,3',5-Triiodo-L-thyronine (T3)	100
L-Thyroxine (L-T4)	0.60
D-Thyroxine (D-T4)	0.64
3,3',5'-Triiodo-L-thyronine (Reverse T3)	2.03
Phenytoin (Phenytoin-D10)	0.003
3,5-Diiodo-L-thyronine (3,5-T2)	1.98
3,5-Diiodo-L-tyrosine dihydrate	<0.001

15.3 Interferences

An interference study was performed according to the CLSI EP07 guideline. No significant interference was observed for concentrations of up to 10 g/L haemoglobin, 15 mg/dL bilirubin conjugated, 30 mg/dL bilirubin unconjugated, 15 mg/mL triglycerides, 1.8 µg/mL HAMAS, 3.6 µg/mL Biotin and 1688 IU/mL Rheumatoid Factor.

15.4 Precision

The precision study was performed according to the CLSI EP5-A3 guideline.
The experimental protocol used a nested components-of-variance design (a 3 x 1 x 5 x 2 x 4 design) with 5 testing days, an automated machine and 2 operators, 1 kit lot, six runs per testing day (two runs per operator or automated machine), and four replicate measurements per run for each sample (total of 120 results per sample). The results were analyzed with a two-way nested ANOVA and summarized in the table below.

Sample	Mean (ng/mL)	SD /CV%	Repeat-ability	Reproduc-ibility	Within Condition	Between Condition	Between Replicate	Between Day
1	0.809	SD	0.08	0.11	0.11	0.03	0.05	0.06
		CV	9.9	13.9	13.4	3.7	5.8	7.1
2	2.147	SD	0.13	0.21	0.21	0.05	0.04	0.16
		CV	5.9	9.9	9.6	2.4	1.6	7.4
3	4.075	SD	0.18	0.39	0.32	0.23	0.15	0.22
		CV	4.4	9.7	7.9	5.6%	3.6	5.5
4	1.110	SD	0.08	0.12	0.12	0.04	0.06	0.06
		CV	7.3	11.2	10.6	3.6	5.3	5.5
5	2.100	SD	0.13	0.17	0.14	0.09	0.00	0.05
		CV	6.0	7.9	6.5	4.5	0.0	2.6
6	3.335	SD	0.14	0.22	0.17	0.14	0.07	0.07
		CV	4.1	6.6	5.0	4.3	2.1	2.1
7	0.410	SD	0.06	0.07	0.07	0.02	0.00	0.04
		CV	14.0	17.6	17.2	4.12	0.0	9.9
8	5.962	SD	0.20	0.40	0.29	0.28	0.10	0.18
		CV	3.3	6.7	4.8	4.6	1.7	3.1
9	1.077	SD	0.10	0.14	0.13	0.04	0.05	0.07
		CV	9.1	12.9	12.2	4.2	4.6	6.7
10	1.110	SD	0.09	0.11	0.10	0.05	0.00	0.05
		CV	8.0	10.1	9.0	4.5	0.2	4.1

15.5 Linearity

The linearity study was according to the CLSI EP06-A guideline using three human serum samples covering the range of the assay.
The samples were diluted in calibrator A at several equidistant concentration levels and the regression equation of the results (y) compared to the concentration (x) predicted from the dilution factor was:
y = 0.99x + 0.17, r = 0.99.
The statistical analysis shows that the assay is sufficiently linear up to a 1:5 dilution when using calibrator A as the diluent.

15.6 Recovery

Spiked samples were prepared by adding defined amounts of T3 (1, 3 and 10 ng/mL in calibrator A) to four serum samples at 1:1 by volume.
The results (in ng/mL) are tabulated below:

Sample	Observed Result	Expected Result	Recovery %
1 Unspiked	1.028	-	-
+1 ng/mL	1.027	1.014	101.3
+3 ng/mL	2.052	2.014	101.9
+10 ng/mL	5.266	5.514	95.5
2 Unspiked	1.018	-	-
+1 ng/mL	0.972	1.009	96.3
+3 ng/mL	1.854	2.009	92.3
+10 ng/mL	4.899	5.509	88.9
3 Unspiked	1.285	-	-
+1 ng/mL	1.019	1.143	89.2
+3 ng/mL	2.115	2.143	98.7
+10 ng/mL	4.903	5.643	86.9
4 Unspiked	1.109	-	-
+1 ng/mL	0.887	1.055	84.1
+3 ng/mL	1.772	2.055	86.2
+10 ng/mL	4.838	5.555	87.1

16. REFERENCE RANGES

Reference ranges (95%) were estimated using samples obtained from apparently healthy adult individuals of diverse races. Each laboratory shall establish their own reference values.

Group	N	Median (ng/mL)	95% Confidence Range (ng/mL)
Adult Males & Females	334	1.08	0.76–1.66

17. REFERENCES

1. L. Lorenzini, N. M. Nguyen, G. Sacripanti, et al. Assay of Endogenous 3,5- diiodo-L-thyronine (3,5-T2) and 3,3′-diiodo-L-thyronine (3,3′-T2) in Human Serum: A Feasibility Study. Front Endocrinol (Lausanne). 2019; 10: 88. Published online 2019 Feb 19. doi: 10.3389/fendo.2019.00088
2. J. Favresse, M.C. Burlacu, D. Maiter et al. Interferences with Thyroid Function Immunoassays: Clinical Implications and Detection Algorithm. Endocrine Reviews 39: 830 – 850, 2018.
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4. G. D. Villanger, E. Learner, M. P. Longnecker et al. Effects of Sample Handling and Analytical Procedures on Thyroid Hormone Concentrations in Pregnant Women's Plasma. Epidemiology. 2017 May; 28(3): 365–369. doi:10.1097/EDE.0000000000000606
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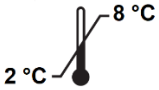
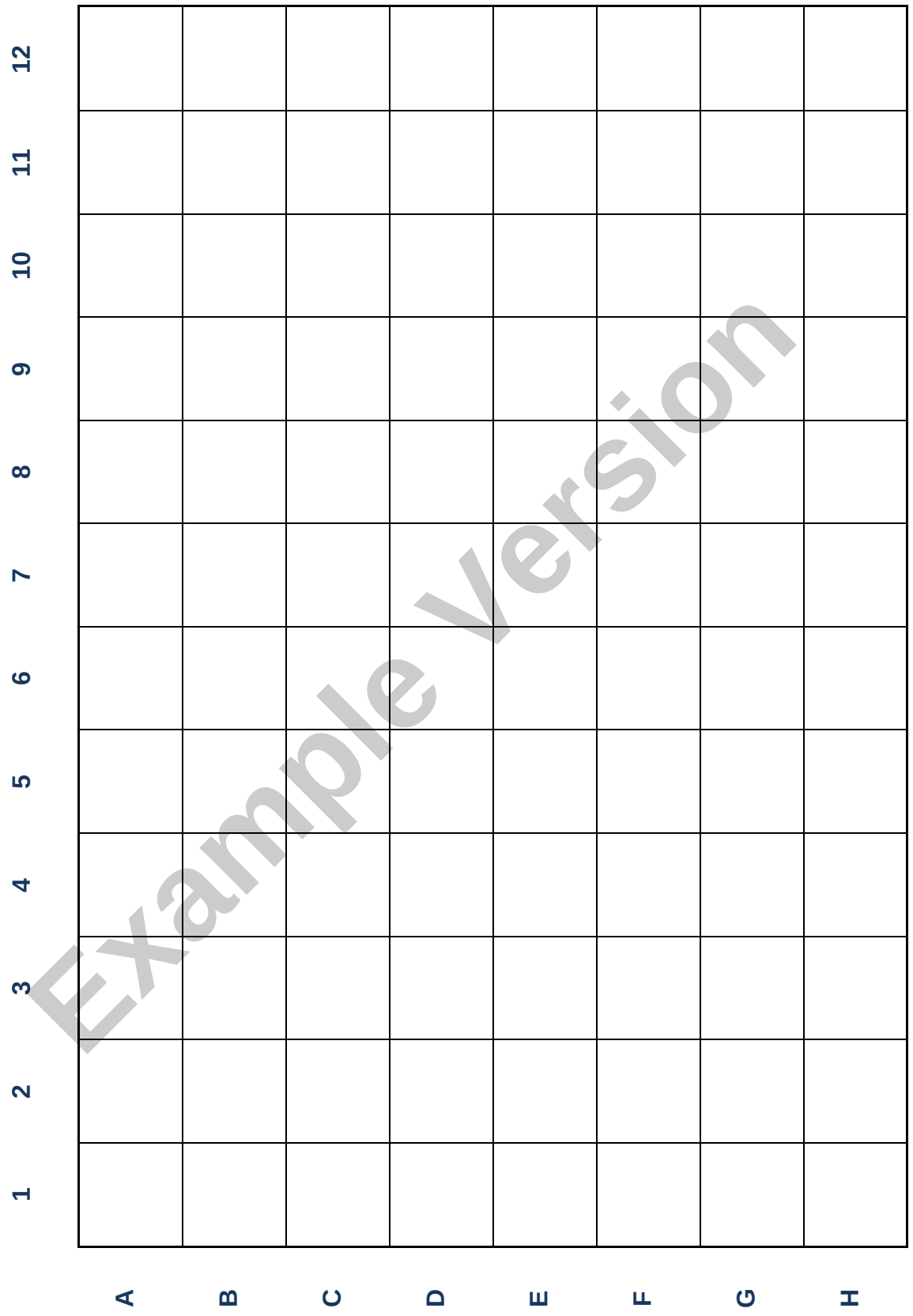
6. K.H. Gless, P. Oster and M. Hüfner. Influence of D-Thyroxine on Plasma Thyroid Hormone Levels and TSH Secretion. Horm. Metab. Res. 9 (1977) 69-73.
7. D. C. Nelson, R. M. Weiss, J. E. Lewis, et al. A Multiple Ligand-Binding Radioimmunoassay of Diiodotyrosine. The Journal of Clinical Investigation Volume 53 February 1974: 416-422.
8. P. R. Larsen. Direct Immunoassay of Triiodothyronine in Human Serum. J Clin Invest. 1972;51(8):1939-1949. https://doi.org/10.1172/JCI107000
9. P. R. Larsen. Inhibition of Triiodothyronine (T3) Binding to Thyroxine binding Globulin by Sodium Salicylate and its Application to Immunoassay of T3 in Human Serum. METABOLISM, Vol. 20, No. 10, 1971.
10. https://www.uofmhealth.org/health-library/hw27377
11. https://labtestsonline.org/tests/phenytoin

18. 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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