EIASON® anti - Tg

IVD

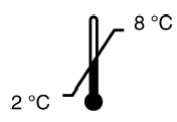
Enzymeimmunoassay for the quantitative determination of autoantibodies against Thyroglobulin (Tg) in human serum and plasma

Kit instruction

For in-vitro use only

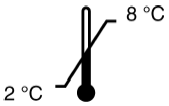
Product of

IASON GmbH
Feldkirchner Straße 4
A – 8054 Graz-Seiersberg
Tel.: +43 (0)316 28 43 00
Fax: +43 (0)316 28 43 00-4
Email: office@iason.eu
www.iason.eu



REF E01-011-96

Used IFU symbols

Symbol	English	Symbol	English
IVD	In vitro diagnostic device	PROTA	Conjugate (protein A conjugated to alkaline peroxidase)
REF	Order number	DIL	Buffer for dilution
	Manufactured by	CAL0-4	Calibrators
	Storage at	CO1 CO2	Control sera
LOT	Lot number	SUB	Substrate
CE	EC Declaration of conformity	STOP	Stop solution
	Expiry date	WASH	Wash solution
MPL	Microplate		

Intended use

For in-vitro use only.

The EIASON® anti – Tg it is an enzymeimmunoassay intended for the quantitative determination of autoantibodies against thyroglobulin in human serum. The results of the measurement of autoantibodies against thyroglobulin are to be used in conjunction with other clinical and laboratory data to assist the clinician in the diagnosis of autoimmune thyroid disease.

Assay principle

The EIASON® anti – Tg is a solid phase immunoenzymometric assay [IEMA] for the quantitative determination of autoantibodies against thyroglobulin in human serum. The assay is based on an easy to use and flexible strip well system (microplate) employing wells coated with highly purified thyroglobulin.
Thyroglobulin autoantibodies in human serum are allowed to bind onto the coated microwells. Bound autoantibodies against thyroglobulin are allowed to interact with protein A conjugated to alkaline peroxidase. The amount of autoantibodies against thyroglobulin (anti Tg) is detected by addition of pNPP (*para*-nitrophenyl phosphate) substrate and stopping the reaction after an incubation time of about 15 minutes by NaOH solution. The absorbance of the yellow reaction mixture is then read at 405nm using an ELISA plate reader. A higher absorbance indicates the presence of TgAb in the test sample. The measuring interval is 65 – 5000 u/mL (NIBSC 65/093).

Warnings and precautions

The EIASON® anti – Tg kit is for in vitro diagnostic use only and is not for internal use in humans or animals. This product must be used strictly in accordance with the instructions set out in the Package Insert. IASON will not be held responsible for any loss or damage (except as required by statute) caused, arising out of non-compliance with the instructions provided.
CAUTION: this kit contains material of human and/or animal origin. Handle kit reagents as if capable of transmitting an infectious agent.
Source material from Human origin which is used in this kit was tested and found negative for HbsAG and HIV as well as for HCV antibodies. However, since there is no diagnostic procedure that excludes these agents with 100 percent certainty all components should be handled as potentially hazardous material.
Appropriate precautions and good laboratory practices must be used in the storage, handling and disposal of the kit reagents. Disposal of kit reagents should be in accordance with local regulations.

Shelf life and storage of reagents

This kit is stable until the stated expiry date if stored as specified. Upon receipt, store all reagents at 2-8°C.

Storage and preparation of serum samples

Sera have to be isolated by centrifugation and should be assayed soon after separation or stored, preferably in aliquots, at or below -20°C. Repeated freezing and thawing or increases in storage temperature must be avoided. Incorrect storage of serum samples can lead to loss of anti - Tg activity. When required, thaw test sera at room temperature and mix gently to ensure homogeneity.
Do not use lipaemic or grossly haemolysed serum samples.
EDTA, citrate and heparin plasma may be used in the assay.

Centrifuge serum prior to assay (preferably for 5 min at 10,000-15,000 x g in a microfuge) to remove particulate matter. Please do not omit this centrifugation step if sera are cloudy or contain particulates.

Materials provided

Allow all reagents A-I to reach room temperature before use.

A. MPL Microplate coated with highly purified Tg (96 wells in total, 8 wells per strip). Before opening the packet of the microplate, allow it to stand at room temperature (20 - 25°C) for at least 30 minutes. After opening, keep any unused strips in the original foil packet with the desiccant provided (reseal with adhesive tape). Store at 2 - 8°C until expire date.

B. CAL 0 - 4 Calibrators (1 mL each); ready to use:
0, 35, 150, 1000, 5000 U/mL (units are NIBSC 65/093).

C. CO1 CO2 Control 1 and Control 2 concentration see QC-Certificate (0.5 mL each); concentrated dilute 1:20 with DIL.

D. DIL for dilution of serum samples and CO1, CO2, 125 mL, ready to use.

E. PROTA (11 mL); ready to use.

F. SUB pNPP (*para* - nitrophenyl phosphate); ready to use (11 mL).

G. STOP NaOH, ready to use (10.5 mL).

H. WASH (125 mL), dilute 1:10 with distilled water. Store at 2-8°C until expire date.

Materials required but not provided in the kit

- Pipettes capable of dispensing 50 µL, 100 µL, 20 µL and 1 - 10 mL
- Distilled water
- ELISA plate reader suitable for 96 well formats and capable of measuring absorbances at 405 nm

Assay procedure

Calculate the number of individual MPL wells needed for the assay. Allow all the reagents supplied including the appropriate number of strips to reach room temperature. After opening, keep any unused strips in the original foil packet (reseal with adhesive tape) with the desiccant provided. Store the unused strips at 2-8°C. Kit controls should always be included in each assay run.

Please note that the optical density depends on incubation time and temperature. Therefore, it is necessary to bring all reagents to work-ready state before the start of the assay; the caps should be opened and all required wells should be in strip holder. Only such preparation will ensure equal elapsed time for each pipetting step without interruption.

Each run must include a standard curve and controls.

1. Dilute all CO1, CO2 and all test samples 1:20 with DIL.
2. Pipette 50 µL of CAL 0-4, CO1, CO2 and test sera (in duplicates) into the wells of MPL.
3. Cover the frame and shake the plate containing the various samples for 15 minutes at 20-25° on an ELISA plate shaker (500 rpm).
4. After the 15 minutes incubation (point 3), discard the samples by briskly inverting the frame of strip wells over a suitable receptacle. Wash 3 times with 350 µL WASH per well and each time tap the inverted wells gently on a clean dry absorbent surface to remove any droplets of WASH.
5. Pipette very carefully 100 µL of reconstituted PROTA into each well.
6. Incubate for 15 min at 20-25°C with shaking (500 rpm).
7. After the 15 minutes incubation with PROTA, discard the PROTA by briskly inverting the wells over a suitable receptacle, wash 3 times with WASH as described under point 5.
8. Pipette very carefully 100 µL of SUB (TMB) into each well.
9. Incubate for 15 minutes at 20-25°C in the dark during which time a blue colour will develop.
10. Stop the substrate reaction by careful addition of 100 µL of STOP to each well (this will cause the blue colour to turn yellow). It is most important to ensure that the substrate incubation time (i.e. time from addition of SUB to addition of STOP) is the same for each well.
11. Measure the absorbance of each well at 405 nm using a microplate reader within 30 minutes after adding the stop solution.

or automated on:

- **IASON® Quardette**
- **IASON® PersonalLab**
- **IASON® Gladiator**

Quality control

The regular use of control samples at several analyte levels is advised to ensure day-to-day validity of results. Two kit controls are provided. The controls should be tested as unknowns. Quality Control charts should be maintained to follow the assay performance.
The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.
It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.
Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.
After checking the above mentioned items without finding any error contact your distributor or IASON directly.

Calculation of results

- Draw average of CAL 0-4 absorbance against concentration lin/log and draw a standard curve
- Lin-log algorithm curve fit is recommended as alternative data reduction
- Serum samples of concentration greater than concentration of CAL 4 can be further diluted with DIL to bring them within the measuring interval of the assay.

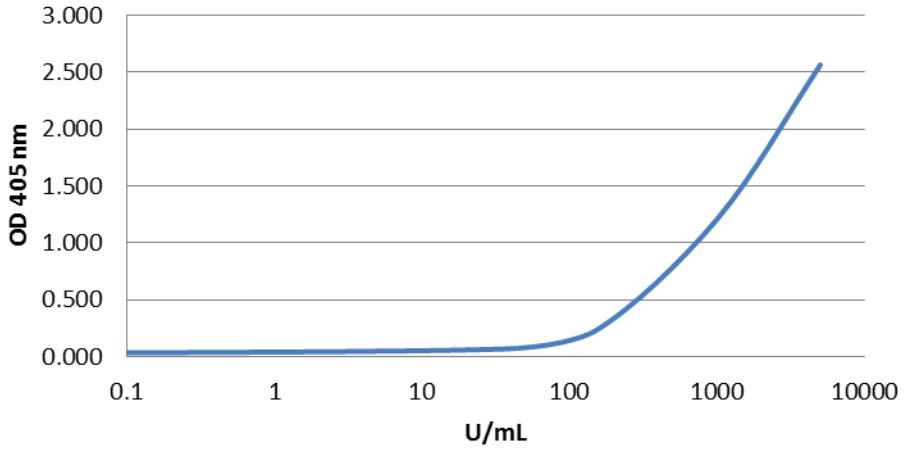
Sample assay data

Typical results of EIASON® anti-Tg Calibrators:

sample	OD (405 nm)
CAL0 = 0 U/mL	0.024
CAL1 = 35 U/mL	0.069
CAL2 = 150 U/mL	0.231
CAL3 = 1000 U/mL	1.212
CAL4 = 5000 U/mL	2.567

This data is for illustration only and must not be used for the calculation of any sample result.

Typical calibration curve



This data is for illustration only and must not be used for the calculation of any sample result.

Reference values

	NIBSC 65/93
	U/mL
Negative	< 65
Positive	≥65

Each laboratory is recommended to determine ranges for their local population.

CLINICAL EVALUATION

Clinical specificity

Sera from 200 individual healthy blood donors were assayed in the EIASON® anti-Tg kit. 190 (95%) were identified as being negative for TgAb.

Clinical sensitivity

Sera from 66 patients diagnosed with Hashimoto's or Graves` diseas were assayed in the EIASON® anti-Tg kit. 48 (73%) were identified as being positive for TgAb.

Lower detection limit

The zero calibrator CAL 0 was assayed 20 times and the mean and standard deviation calculated. The lower detection limit at 2 standards deviations was 9.8 U/mL.

Precision

Inter assay precision			Inter assay precision		
Sample	Mean U/mL (n=20)	CV (%)	Sample	Mean U/mL (n=25)	CV (%)
1	56	15.8	A	61	7.1
2	205	10.9	B	139	4.6
3	759	7.2	C	711	2.8
4	1864	7.3	D	1315	3.3

Clinical accuracy

Analysis of sera from patients with autoimmune diseases other than Graves' or Hashimoto's diseases indicated no interference from antibodies for 21-OH (n=4). 29% (n=7) of sera positive for antibodies to GAD, 18% (n=17) of sera positive for antibodies to IA-2, 44% (n=9) of sera positive for acetylcholine receptor, 40% (n=5) of sera positive for antibodies to dsDNA and 25% (n=28) of sera positive for Rheumatoid Factor were positive for TgAb in the EIASON® anti-Tg.

Interference

No interference was observed was observed when samples were spiked with the following materials; haemoglobin up to 500 mg/dL, bilirubin up to 20 mg/dL and intralipid up to 3000 mg/dL.

LEGAL ASPECTS

Reliability of results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.
The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact IASON.

Therapeutic consequences

Therapeutic consequences should never be based on laboratory results only even if all test results are in agreement with the items as stated under Reliability of Results. Any laboratory result is only a part of the total clinical picture of a patient.
Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.
The test result itself should never be the sole determinant for deriving any therapeutic consequences.

Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point Therapeutic Consequences are also invalid. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

Literature

1. Rees Smith B. Thyroid autoantibodies. Scand. J. Clin. Lab. Invest. (2001) 61 (Suppl. 235): 45-52.
2. Burne P. et al. Point-of-care assays for autoantibodies to thyroid peroxidase and to thyroglobulin. Thyroid 2005, 15: 1005-1010.

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Pipetting scheme

Allow all reagents to reach room temperature before use.

1. Preparation of the CO1, CO2 and sera: dilute all samples 1:20 with **DIL**:

1 A. Pipetting **CO1** **CO2** /sera	25 µL
1 B. Pipetting **DIL**	475 µL

2. Test procedure

2.Pipetting	**CAL 0-4** **50 µL**	**CO1, CO2** **50 µL**	Sample **50 µL**
3.Incubation	15 min at room temperature (20 - 25°C) on a shaker (500 rpm)		
4.Washing	wash 3 times: aspirate or decant add 350 µL **WASH** repeat wash step 2 x and dry on absorbant material		
5.Pipetting	**PROTA** **100 µL**		
6.Incubation	15 min at room temperature (20-25°C) on a shaker (500 rpm)		
7.Washing	see step 4		
8.Pipetting	**SUB** **100 µL**		
9.Incubation	15 minutes at room temperature in the dark (20 - 25°C)		
10.Pipetting	**STOP** **100µL**		
11.Reading	at 405 nm Calculation: lin-log		

Expected Values

	NIBSC 65/093
	U/mL
Negative	< 65
Positive	≥65