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AESKULISA®

THE DIAGNOSTIC TOOL THAT WORKS

INSTRUCTION MANUAL

AESKULISA® Tg

Ref 3402

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Instruction Manual

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1 Intended Use

***AESKULISA®* Tg** is an indirect solid phase enzyme immunoassay for the quantitative detection of thyroglobulin (Tg) in human serum. The assay employs selected specific monoclonal antibodies against human thyroglobulin (Tg).

The assay is a tool in the follow up and monitoring of thyroid carcinoma as well as for the differential diagnosis of thyroid diseases.

2 Clinical Application and Principle of the Assay

Thyroglobulin (Tg) is a glycoprotein of high molecular weight (660kDa) localized within the colloid of the thyroid follicle. It plays an essential role in the storage of iodine and acts as substratum for the synthesis of iodinated thyroid hormones thyroxine (T4) and 3,5,3`-triiodothyronine (T3).

Elevated thyroglobulin serum concentrations have been reported in various thyroid diseases, such as, hyperthyroidism, non-toxic goiter, thyroiditis and differenciated thyroid carcinoma.

The main indication of the Tg determination, however is the postoperative monitoring of differentiated thyroid carcinoma. Its clinical value is the early detection and exclusion of metastases or tumor relapses and the follow-up of radioiodine treatments. Serum Tg is non detectable in patients who underwent total thyroidectomy including ablation by radioiodine and are free of metastases and tumor. These patients in true complete remission will not display Tg levels, even by endogenous TSH stimulation.

Consequently detectable Tg values in these group of patients are an important indication for still existing or newly developed neoplasia. Particularly if these detectable Tg values are increasing under a TSH-suppressive thyroid hormone treatment (Tg profiles).

In contrast, thyroglobulin levels in patients with medullary carcinoma or undifferentiated tumors remain within the normal range. Since thyroglobulin levels could also be elevated in other benign thyroid diseases, this test is not a criteria for the diagnosis of malignant thyroid tumors.

Determination of thyroglobulin is of prognostic value in Graves` disease patients undergoing therapy.

Significantly elevated Tg levels at the end of a thyrostatic therapy are indication for a higher risk of relapse, whereas patients with continuous low thyroglobulin concentrations tend to continual recovery

Principle of the test

Undiluted serum samples are incubated in the microplates coated with monoclonal antibodies against human thyroglobulin (Tg). Tg, if present in the specimen, bind to the antibodies. The unbound fraction is washed off in the following step. Afterwards monoclonal anti-Tg immunoglobulins conjugated to horseradish peroxidase (conjugate) are incubated and react with the antigen-antibody complex of the samples in the microplates. Unbound conjugate is washed off in the following step. Addition of TMB-substrate generates an enzymatic colorimetric (blue) reaction, which is stopped by diluted acid (color changes to yellow). The rate of color formation from the chromogen is a function of the amount of conjugate bound to the antigen-antibody complex and this is proportional to the initial concentration of Tg in the patient sample.

3 Kit Contents

TO BE RECONSTITUTED				
Item	Quantity	Cap color	Solution color	Description / Contents
Sample Buffer (5x)	1 x 20ml	White	Yellow	5 x concentrated Tris, sodium chloride (NaCl), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Wash Buffer (50x)	1 X 20ml	White	Green	50 x concentrated Tris, NaCl, Tween 20, sodium azide < 0.1% (preservative)
READY TO USE				
Item	Quantity	Cap color	Solution color	Description / Contents
Negative Control	1 x 1.5ml	Green	Colorless	Control material (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
10 ng Control	1 x 1.5ml	Red	Yellow	Control material (diluted), bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Tg Recovery	2 x 1.8ml	Blue	Yellow	Thyroglobulin, bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Calibrators	5 x 1.5ml	White	Yellow *	Concentration of each calibrator: 3.75, 7.5, 15, 30, 60 ng/ml. Thyroglobulin, bovine serum albumin (BSA), sodium azide < 0.1% (preservative)
Conjugate, anti-Tg	1 x 15ml	White	Green	Monoclonal anti-Tg antibodies conjugated to horseradish peroxidase, bovine serum albumin (BSA)
TMB Substrate	1 x 15ml	Black	Colorless	Stabilized tetramethylbenzidine and hydrogen peroxide (TMB/H_2O_2)
Stop Solution	1 x 15ml	White	Colorless	1M Hydrochloric Acid
Microtiter plate	12 x 8 well strips	N/A	N/A	With breakaway microwells. Refer to paragraph 1 for coating.

* Color increasing with concentration

MATERIALS REQUIRED, BUT NOT PROVIDED

Microtiter plate reader 450 nm reading filter and recommended 620 nm reference filter (520-690 nm). Glass ware (cylinder 100-1000ml), test tubes for dilutions. Vortex mixer, precision pipettes (10, 100, 200, 500, 1000 µl) or adjustable multipipette (100-1000µl). Microplate washing device (300 µl repeating or multichannel pipette or automated system), adsorbent paper. Our tests are designed to be used with purified water according to the definition of the United States Pharmacopeia (USP 26 - NF 21) and the European Pharmacopeia (Eur.Ph. 4th ed.).

4 Storage and Shelf Life

Store all reagents and the microplate at 2-8 °C/35.6-46.4 °F, in their original containers. Once prepared, reconstituted solutions are stable at 2-8 °C/35.6-46.4 °F for 1 month. Reagents and the microplate shall be used within the expiry date indicated on each component, only. Avoid intense exposure of TMB solution to light. Store microplates in designated foil, including the desiccant, and seal tightly.

5 Precautions of Use

5.1 Health hazard data

THIS PRODUCT IS FOR IN VITRO DIAGNOSTIC USE ONLY. Thus, only staff trained and specially advised in methods of in vitro diagnostics may perform the kit. Although this product is not considered particularly toxic or dangerous in conditions of the intended use, refer to the following for maximum safety:

Recommendations and precautions

This kit contains potentially hazardous components. Though kit reagents are not classified being irritant to eyes and skin we recommend to avoid contact with eyes and skin and wear disposable gloves.

WARNING ! Calibrators, Controls and Buffers contain sodium azide (NaN_3) as a preservative. NaN_3 may be toxic if ingested or adsorbed by skin or eyes. NaN_3 may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. Please refer to decontamination procedures as outlined by CDC or other local/national guidelines.

Do not smoke, eat or drink when manipulating the kit. Do not pipette by mouth.

All biological source material used for some reagents of this kit has been tested by approved methods and found negative for HbsAg, Hepatitis C and HIV 1. However, no test can guarantee the absence of viral agents in such material completely. Thus handle these as if capable of transmitting infectious diseases and according to national requirements.

The kit contains material of animal origin as stated in the table of contents, handle according to national requirements.

5.2 General directions for use

Do not mix or substitute reagents or microplates from different lot numbers. This may lead to variations in the results.

Allow all components to reach room temperature (20-32 °C/68-89.6 °F) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test.

Incubation: We recommend test performance at 30 °C/86 °F for automated systems.

Never expose components to higher temperature than 37 °C/98.6 °F.

Always pipette substrate solution with brand new tips only. Protect this reagent from light. Never pipette conjugate with tips used with other reagents prior.

A definite clinical diagnosis should not be based on the results of the performed test only, but should be made by the physician after all clinical and laboratory findings have been evaluated.

The diagnosis is to be verified using different diagnostic methods.

6 Sample Collection, Handling and Storage

Use preferentially freshly collected serum samples. Blood withdrawal must follow national requirements. Do not use icteric, lipemic, hemolysed or bacterially contaminated samples. Sera with particles should be cleared by low speed centrifugation (<1000 x g). Blood samples should be collected in clean, dry and empty tubes.

After separation, the serum samples should be used during the first 8 h, respectively stored tightly closed at 2-8 °C/35.6-46.4 °F up to 48 h, or frozen at -20 °C/-4 °F for longer periods. (Thomas: Labor und Diagnose; CLSI Guideline GP44-A4)

7 Assay Procedure

7.1 Preparations prior to starting

Dilute concentrated reagents:

Dilute the concentrated sample buffer 1:5 with distilled water (e.g. 20 ml plus 80 ml).

Dilute the concentrated wash buffer 1:50 with distilled water (e.g. 20 ml plus 980 ml).

To avoid mistakes we suggest to mark the cap of the different calibrators.

Washing:

Prepare 20 ml of diluted wash buffer (1x) per 8 wells or 200 ml for 96 wells

e.g. 4 ml concentrate plus 196 ml distilled water.

Automated washing:

Consider excess volumes required for setting up the instrument and dead volume of robot pipette.

Manual washing:

Discard liquid from wells by inverting the plate. Knock the microwell frame with wells downside vigorously on clean adsorbent paper. Pipette 300 µl of diluted wash buffer into each well, wait for 20 seconds. Repeat the whole procedure twice again.

Microplates:

Calculate the number of wells required for the test. Remove unused wells from the frame, replace and store in the provided plastic bag, together with desiccant, seal tightly (2-8 °C/35.6-46.4 °F).

7.2 Pipetting Scheme

We suggest pipetting calibrators, controls and samples as follows:

For *QUANTITATIVE* interpretation

	1	2	3	4...
A	**CalA**	**CalE**	**P1 + SB**	**P1 + RC**
B	**CalA**	**CalE**	**P2 + SB**	**P2 + RC**
C	**CalB**	**10ng control + SB**	**P3 + SB**	**P3 + RC**
D	**CalB**	**10ng control + SB**	**...**	**...**
E	**CalC**	**NC + SB**	**...**	**...**
F	**CalC**	**NC + SB**	**...**	**...**
G	**CalD**		**...**	**...**
H	**CalD**		**...**	**...**

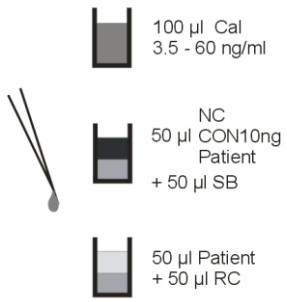
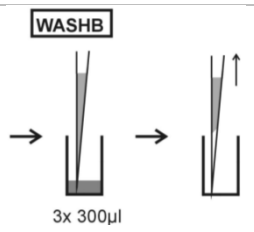
CalA: calibrator 3,75ng
CalB: calibrator 7,5ng
CalC: calibrator 15ng
CON 10ng + SB: 50µl 10 ng control + 50µl sample buffer

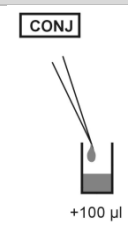
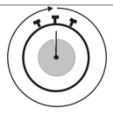
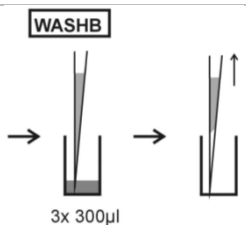
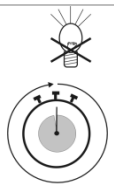
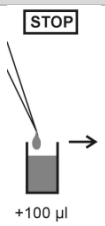
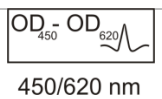
CalD: calibrator 30ng
CalE: calibrator 60ng
Con 10ng: control 10ng
NC + SB: 50µl negative control + 50µl sample buffer

SB: sample buffer
NC: negative control
RC: Tg Recovery
P1 + SB: 50 µl patient`s sample + 50µl sample buffer

P1: patient 1
P2: patient 2
P3: patient 3
P1 + RC: 50µl patient`s sample + 50µl recovery

7.3 Test Steps

Step	*Description*
1.	Ensure preparations from step 7.1 above have been carried out prior to pipetting.
2.	Use the following steps in accordance with quantitative interpretation results desired:
	CONTROLS & SAMPLES
3.	 Patient samples should be tested with and without Tg Recovery reagent and therefore need to be run in duplicate. a. Pipette 100µl of the each calibrator into its relevant well. b. Pipette 50µl of the negative control, 10ng control and each patient sample into separate wells. c. Pipette 50µl of sample buffer into the negative control, 10 ng control and each of the patient sample wells, without TgRecovery reagent. • For patient results with TgRecovery, add 50µl of TgRecovery reagent to the patient samples. Shake carefully.
4.	 Incubate for 60 minutes at 20-32 °C/68-89.6 °F.
5.	 Wash 3x with 300 µl washing buffer (diluted 1:50).

	CONJUGATE	
6.		Pipette 100 µl conjugate into each well.
7.		Incubate for 60 minutes at 20-32 °C/68-89.6 °F.
8.		Wash 3x with 300 µl washing buffer (diluted 1:50).
	SUBSTRATE	
9.		Pipette 100 µl TMB substrate into each well.
10.		Incubate for 60 minutes at 20-32 °C/68-89.6 °F, protected from intense light.
	STOP	
11.		Pipette 100 µl stop solution into each well, using the same order as pipetting the substrate.
12.		Incubate 5 minutes minimum.
13.		Agitate plate carefully for 5 sec.
14.		Read absorbance at 450 nm (recommended 450/620 nm) within 30 minutes.

8 Quantitative Interpretation

Establish the standard curve by plotting the optical density (OD) of each calibrator (y-axis) with respect to the corresponding concentration values in ng/ml (x-axis). For best results use linear regression with log-log coordinates for optical density and concentration (both logarithmic scale). From the OD of each sample, read the corresponding Tg concentrations expressed in ng/ml.

Example of a standard curve

We recommend pipetting calibrators in parallel for each run.

Calibrators Tg	OD 450/620 nm	CV %
3.75 ng/ml	0.232	2.7
7.5 ng/ml	0.440	3.9
15.0 ng/ml	0.805	2.6
30.0 ng/ml	1.436	0.68
60.0 ng/ml	2.444	3.2

Do not use this example for interpreting patients results!

Samples above the highest calibrator range should be reported as >Max. They should be diluted as appropriate and re-assayed. Samples below calibrator range should be reported as < Min.
For lot specific data, see enclosed quality control leaflet. Medical laboratories might perform an in-house quality control by using own controls and/or internal pooled sera, as foreseen by national regulations.

Each laboratory should establish its own normal range based upon its own techniques, controls, equipment and patient population according to their own established procedures.

In case that the values of the controls do not meet the criteria the test is invalid and has to be repeated.

The following technical issues should be verified: Expiration dates of (prepared) reagents, storage conditions, pipettes, devices, photometer, incubation conditions and washing methods.

If the items tested show aberrant values or any kind of deviation or that the validation criteria are not met without explicable cause please contact the manufacturer or the supplier of the test kit.

Recovery test

Anti-Tg antibodies or unspecific effects in a patient's serum may interfere with serum thyroglobulin assays. Consequently, sera should be tested for such interferences by carrying out a recovery test as follows.

In parallel to the original patient sample add 50 µl of Tg Recovery to 50 µl of the serum of investigation.

Recovery (in %) in the serum sample is calculated as stated below:

$$\frac{\text{ng Tg/ml (PR1)} - \text{ng Tg/ml (P1)}}{\text{ng Tg/ml (C)}} \times 100 = \%\ \text{Recovery}$$

P1: Patient result without Tg recovery
PR1: Patient result with Tg recovery
C: 10 ng Control

Given unimpaired recovery (100 %), (e.g. no factors are present in the patient's serum that interfere with Tg determination), the result shall be approximately 10 ng/ml above the Tg level of the corresponding original sample. Taking into consideration pipetting inaccuracies, recoveries between 70 % and 130 % are considered valid. Levels of < 70 % or > 130 % are due to interference and the Tg level of the relevant original sample has to be considered invalid.

The concentration for Tg Recovery is included in the enclosed quality control leaflet and is approximately 10 ng Tg/ml.

Do not use QC data for calculation.

Interpretation

Positive results should be verified concerning the entire clinical status of the patient. Also every decission for therapy should be taken individually. It is recommended that each laboratory establishes its own normal and pathological ranges of serum Tg.

9 Technical Data

Sample material:	serum
Sample volume:	100 µl of sample, undiluted
Total incubation time:	180 minutes at room temperature (20-32 °C/68-89.6 °F)
Calibration range:	3.75 - 60 ng/ml
Analytical sensitivity:	3.75 ng/ml
Storage:	at 2-8 °C/35.6-46.4 °F use original vials, only
Number of determinations:	96 tests

10 Performance Data

10.1 Functional assay sensitivity

The functional sensitivity of this kit has been found at 3.75 ng/ml.

10.2 Specificity

The microplate is coated with monoclonal antibodies highly specific for Tg. No crossreactivities to other antigens have been found.

10.3 Linearity

Chosen sera have been tested with this kit and found to dilute linearly. However, due to the heterogeneous nature of human autoantibodies there might be samples that do not follow this rule.

10.4 Calibration

In the absence of an international reference standard, the quantitative measurement system is calibrated in arbitrary units. The results are expressed in ng/ml.

10.5 ”High dose hook” effect

Concentrations of up to 100,000 ng Tg/ml did not result in a ”high dose hook” effect.

10.6 Interference with autoantibodies

Chosen seras with various levels of anti-Tg have been spiked with Tg. No effect on results has been observed. However, this does not mean that all patient seras follow these results. Thus recovery tests should be performed always.

11 Literature

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CLSI Guideline GP44-A4: Procedures for the Handling and Processing of Blood Specimens for Common Laboratory Tests

12 Regulatory Symbols

IVD	- Diagnosi in vitro	- For in vitro diagnostic use
	- Pour diagnostic in vitro	- Para uso diagnóstico in vitro
	- In Vitro Diagnostikum	- In Vitro Διαγνωστικό μέσο
	- Para uso Diagnóstico in vitro	
REF	- Numero d'ordine	- Cataloge number
	- Référence Catalogue	- Numéro de catálogo
	- Bestellnummer	- Αριθμός παραγγελίας
	- Número de catálogo	
LOT	- Descrizione lotto	- Lot
	- Lot	- Lote
	- Chargen Bezeichnung	- Χαρακτηρισμός παρτίδας
	- Lote	
UDI	- Identificatore univoco del dispositivo	- Unique Device Identifier
	- Identifiant unique de l'appareil	- Identificador único del dispositivo
	- eindeutige Produktidentifizierung	- Μοναδικό αναγνωριστικό συσκευής
	- Identificador único do dispositivo	
CE	- Conformità europea	- EC Declaration of Conformity
	- Déclaration CE de Conformité	- Declaración CE de Conformidad
	- Europäische Konformität	- Ευρωπαϊκή συμφωνία
	- Déclaracão CE de Conformidade	
Σ 96	- 96 determinazioni	- 96 tests
	- 96 tests	- 96 pruebas
	- 96 Bestimmungen	- 96 προσδιορισμοί
	- 96 Testes	
eIFU WWW.AESKU.COM	- Rispettare le istruzioni elettroniche per l'uso	- See electronical instructions for use
	- Voir les instructions d'utilisation électronique	- Siga las instrucciones electrónicas de uso
	- Elektronische Gebrauchsanweisung beachten	- Ακολουθήστε τις ηλεκτρονικές οδηγίες χρήσης
	- Seguir as instruções electrónicas de utilização	
	- Da utilizzarsi entro	- Use by
	- Utilise avant le	- Utilizar antes de
	- Verwendbar bis	- Χρήση μέχρι
	- Utilizar antes de	
+2°C +8°C	- Conservare a 2-8°C (35.6-46.4°F)	- Store at 2-8°C (35.6-46.4°F)
	- Conserver à 2-8°C (35.6-46.4°F)	- Conservar a 2-8°C (35.6-46.4°F)
	- Lagerung bei 2-8°C (35.6-46.4°F)	- Φυλάσσεται στους 2-8°C (35.6-46.4°F)
	- Conservar entre 2-8°C (35.6-46.4°F)	
	- Prodotto da	- Manufactured by
	- Fabriqué par	- Fabricado por
	- Hergestellt von	- Κατασκευάζεται από
	- Fabricado por	
CON 10ng	- Controllo 10 ng	- Positive 10 ng
	- Contrôle 10 ng	- Control 10 ng
	- Kontrolle 10 ng	- 10 ng ορός ελέγχου
	- Controlo 10 ng	
CON –	- Controllo negativo	- Negative Control
	- Contrôle Négatif	- Control Negativo
	- Negativ Kontrolle	- Αρνητικός ορός ελέγχου
	- Controlo negativo	
CAL	- Calibratore	- Calibrator
	- Etalon	- Calibrador
	- Kalibrator	- Αντιδραστήριο βαθμονόμησης
	- Calibrador	
CONJ	- Coniugato	- Conjugate
	- Conjugé	- Conjugado
	- Konjugat	- Σύζευγμα
	- Conjugado	
MP	- Micropiastra rivestita	- Coated microtiter plate
	- Microplaque sensibilisée	- Microplaca sensibilizada
	- Beschichtete Mikrotiterplatte	- Επικαλυμμένη μικροπλάκα
	- Microplaca revestida	
WASHB 50x	- Tampone di lavaggio	- Wash buffer
	- Tampon de Lavage	- Solución de lavado
	- Waschpuffer	- Ρυθμιστικό διάλυμα πλύσης
	- Solucão de lavagem	
SUB	- Tampone substrato	- Substrate buffer
	- Substrat	- Tampón sustrato
	- Substratpuffer	- Ρυθμιστικό διάλυμα υποστρώματος
	- Substrato	
STOP	- Reagente bloccante	- Stop solution
	- Solution d'Arrêt	- Solución de parada
	- Stopreagenz	- Αντιδραστήριο διακοπής αντίδρασης
	- Solucão de paragem	
SB 5x	- Tampone campione	- Sample buffer
	- Tampon Echantillons	- Tampón Muestras
	- Probenpuffer	- Ρυθμιστικό διάλυμα δειγμάτων
	- Diluente de amostra	
RC	¨ Recupero	¨ Recovery
	¨ Corrélation	¨ Recuperado
	¨ Wiederfindung	¨ Ανάκτηση
	¨ Recuperacão	