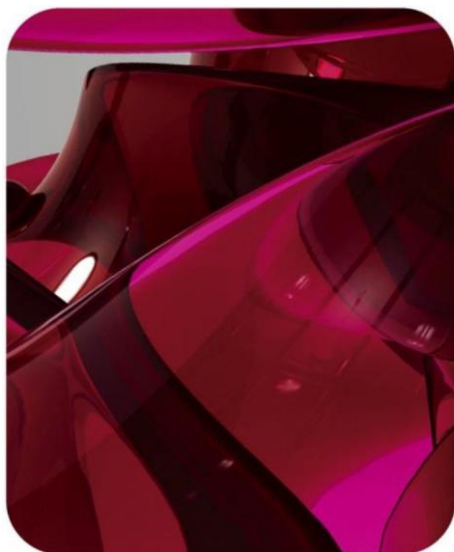




AESKU.DIAGNOSTICS

THE DIAGNOSTIC TOOL THAT WORKS



AESKUBLOTS®

THE DIAGNOSTIC TOOL THAT WORKS

INSTRUCTION MANUAL

AESKUBLOTS® Food Intolerance

Ref 431401 + Ref 431401.BULK120,
Ref 431402 + Ref 431402.BULK120,
Ref 431403 + Ref 431403.BULK120,
Ref 431404 + Ref 431404.BULK120,
Ref 431405 + Ref 431405.BULK120,
Ref 431406 + Ref 431406.BULK120,
Ref 431407 + Ref 431407.BULK120





Instruction Manual

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1 Intended Use

AESKUBLOTS® Food Intolerance is a membrane based enzyme immunoassay for quantitative detection of food-specific IgG₄ antibodies in human plasma or serum. Antigens are located as parallel lines at exactly defined positions on a nitrocellulose membrane.

The assay permits a large number of tests to be conducted in parallel over a short period of time. The assay is used to support the diagnostic process in patients suspected to have food intolerances.

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.

2 Clinical Application and Principle of the Test

AESKUBLOTS® Food Intolerance is an in vitro immunoassay for parallel determination of specific IgG₄ antibodies against foods in plasma or serum. The test is used to support the diagnostic process in patients suspected to have food intolerances. The assay permits a large number of tests to be conducted in parallel over a short period of time. It must be performed by qualified professionals familiar with in vitro diagnostic methods. In diagnostic testing for food intolerances, it is not customary to interpret measurements within stringently defined limits. The given reaction to a particular level of sensitisation is very individual and cannot be fixed within precise limits.

Note! *AESKUBLOTS® Food Intolerance* is used to test foods which permit potential cross-reactions to be predicted. This must be considered when evaluating the results, deciding on further diagnostic examinations, and advising the patient.

Principle of the test

The food extracts are applied as lines on a nitrocellulose membrane. Membrane strips with specific foods at exactly defined positions are incubated in serum or plasma samples diluted 1:20. Patient's antibodies, if present in the specimen, bind to the food proteins. The unbound fraction is washed off in the following step. Afterwards, anti-human IgG₄ immunoglobulins conjugated to Horseradish peroxidase are incubated and react with the antigen-antibody complex of the samples. Unbound conjugate is washed off in the following step. After the addition of the TMB-substrate it is converted by an enzymatic reaction to a blue precipitate. The reaction is stopped by distilled water.



3 Kit Contents

TO BE RECONSTITUTED					
Item	Quantity (option 1)	Quantity (option 2)	Cap color	Solution color	Description / Contents
Wash Buffer (20x)	1 x 50 ml	3 x 100 ml	white	colorless	20x concentrated for preparation of 1 L or 2 L Tris buffer, pH 8.0 ± 0.2
READY TO USE					
Item	Quantity (option 1)	Quantity (option 2)	Cap color	Solution color	Description / Contents
Conjugate, IgG ₄	1 x 15 ml	3 x 20 ml	yellow	colorless	anti-human immunoglobulin G ₄ (IgG ₄) conjugated to horseradish peroxidase
TMB Substrate	1 x 15 ml	3 x 20 ml	black	colorless	stabilized TMB/H ₂ O ₂
Membrane strips	24 strips	120 strips	see certificate	N/A	coated food extracts (see certificate)
Tweezers	1 pcs. each	1 pcs. each	N/A	N/A	N/A
Incubation tray	3 pcs.	15 pcs.	N/A	N/A	N/A
MATERIALS REQUIRED, BUT NOT PROVIDED					
Cylinder 1000 ml (for option 1), respectively cylinder 2000 ml (for option 2). For manual performance additionally required: rocking platform, precision pipettes (100, 1000 µl). For result interpretation required: AESKU.SCAN or HELIA®. Our tests are designed to be used with purified water according to the definition of the United States Pharmacopeia and the European Pharmacopeia.					

4 Storage and Shelf Life

Store all reagents and membrane-strips 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 at least six weeks. Reagents and strips shall be used within the expiry date indicated on each respective component. Don't use components after the expiry dates. Avoid intense exposure of TMB solution to the light.

5 Precautions of Use and General Introductions

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.

All kit components are classified according to Regulation (EC) No. 1272/2008 [CLP]. See the Materials and Safety Document (MSDS) for more information on ingredients.

Substances listed on the so-called "Candidate List of Substances of very High Concern (SVHCV) for authorization" of the European Chemicals Agency (ECHA) are not intentional components of this product. It is therefore not to be expected that these substances are contained in amounts ≥ 0.1 % in the product.

Reagents should be stored safely and be inaccessible to children. Avoid contact with skin. Wear suitable protection gloves. Do not smoke, eat or drink while handling the kit.

In particular, the mixture does not contain any substances in concentrations ≥ 0.1 % to be

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classified as PBT or vPvB.

Patient samples should be considered potentially infectious and handled according to national laws. Patient samples and other potentially infectious material should be decontaminated after the test run.

5.2 General directions for use

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.

To differentiate between the various *AESKUBLOTS® Food Intolerance* tests available, a color code comprised of two colored lines is applied above the reference line of the strips. The upper line defines the product group, where green line stands for Food Intolerance. The lower line specifies the test. The complete color code and the food composition of each test are accessible in the lot specific certificate of analysis. In case that the product information, including the labeling, is incorrect please contact the manufacturer or the supplier of the test kit.

Wash buffer may be interchanged between lots and test kits. All other components are specific for each test kit and are not to be interchanged. Do not exchange reagent components with *AESKUBLOTS®* Autoimmune, Allergy or Borrelia diagnostic tests! For handling of conjugate do not use polystyrene vessels. Allow all components to reach room temperature (20 - 32 °C/ 68 - 89.6 °F) before use, mix well and follow the recommended incubation temperature for an optimum performance of the test. Never expose components to higher temperature than 37 °C/ 98.6 °F. Always pipette substrate solution with brand new tips only. Protect substrate solution from light. Never pipette conjugate with tips previously used with other reagents.

A conclusive clinical diagnosis should not be based solely on the results of a single diagnostic procedure. In addition to the in vitro determination of food-specific IgG₄, a thorough medical history should be taken and the various symptoms investigated. Every patient reacts differently. Hence, identical results delivered by the test will not automatically lead to the same diagnosis. Diverse food extracts with similar molecular structures or epitopes can trigger weak or strong cross-reactions which must always be taken into account. Occasionally, negative in vitro results can also be obtained in patients with symptoms which are clearly correlated to contact with certain foods. Sensitisation to foods other than those contained in the *AESKUBLOTS® Food Intolerance* test cannot be ruled out. Patients with clinical symptoms and Food Intolerance class 0 reactions to the respective foods should be referred to a specialist for further investigation.

The binding capacities of specific IgG₄ antibodies can vary from food to food. Therefore, identical classes for various foods will not necessarily correspond to the same sIgG₄ content. Reliable and reproducible results are achieved if the test is performed in accordance with the methodological instructions and good laboratory practice. The results obtained with *AESKUBLOTS® Food Intolerance* do not necessarily correlate with sIgG₄ antibody titers obtained by other methodologies.

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6 Sample Collection, Handling and Storage

Use preferentially freshly collected serum or plasma samples. Blood withdrawal must follow national requirements. Do not use icteric, lipemic, hemolysed or bacterially contaminated samples. Sera or plasma 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 samples should be used during the first 8 h. Alternatively, the samples should be stored in tightly closed vials at 2 - 8 °C/ 35.6 - 46.4 °F for up to 14 days, or frozen at -20 °C/ -4 °F for longer periods. Do not use heat inactivated samples. (Thomas: Labor und Diagnose; CLSI Guideline GP44-A4)

7 Assay Procedure

7.1 Preparations prior to starting

All reagents and the test strips must be warmed to room temperature (20 – 32 °C / 68 – 89,6 °F) for at least 30 minutes prior to use. Confirm that no salt crystals have been formed in the wash buffer concentrate. If this happened, dissolve the crystals by slightly warming, room temperature should be enough to dissolve the crystals in the concentrate.

Dilute concentrated wash buffer 1:20 with distilled water (e.g. 950 ml of distilled water plus 50 ml of wash buffer concentrate, respectively 1900 ml of distilled water plus 100 ml of wash buffer concentrate) to obtain a ready-to-use wash buffer.

7.2 Test Steps

Important notes:

Follow exactly this protocol. Make sure that the two components mentioned in the protocol are added to the tray in steps 6 and 9.

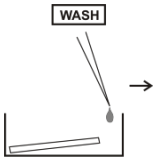
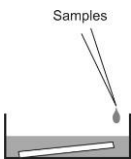
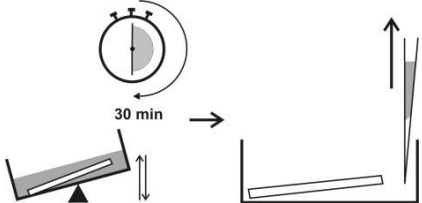
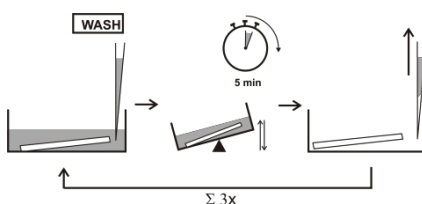
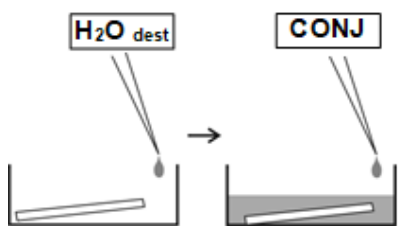
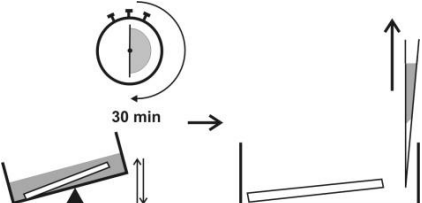
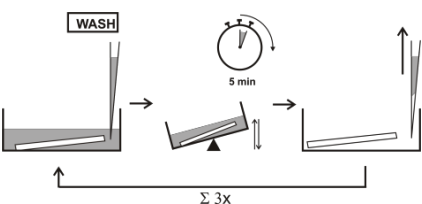
Do not let strip dry out during incubation steps.

Do not touch strip with fingers, use tweezers.

Remove diluted samples completely after incubation of strip to avoid carry over.

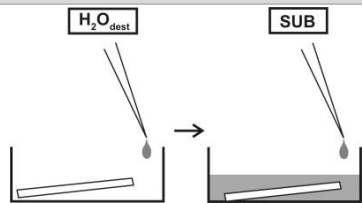
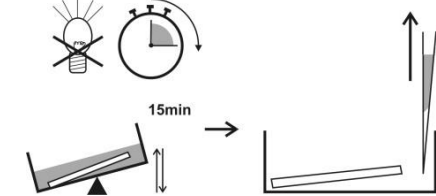
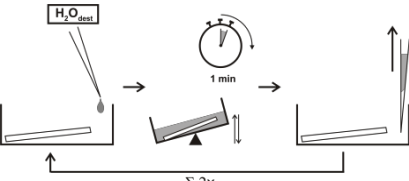
Continuously shake strip during incubation steps with a shaker (angle ≥ 5°).

Dispense sample buffer, conjugate and substrate directly into the diluent (WASH or water) in the incubation tray. Do not allow to flow over the strip.

Step	Description
1.	Ensure the preparations, from step 7.1 above, have been carried out prior to test begin.
2.	 Put strip in correct orientation into incubation tray (reference line and color coding upwards). Put 1425 µl wash buffer into the incubation tray.
CONTROLS & SAMPLES	
3.	 Pipette 75 µl sample into the designated incubation trays with wash buffer.
4.	 Incubate for 30 minutes at 20 - 32 °C/68 - 89.6 °F with agitation. After that remove sample completely.
5.	 Wash 3 times for 5 minutes with 1.8 ml wash buffer by agitation. Remove wash buffer after every washing step.
CONJUGATE	
6.	 Pipette 1050 µl dist. H₂O and 450 µl conjugate into each incubation tray with strip.
7.	 Incubate for 30 minutes at 20 - 32 °C/68 - 89.6 °F with agitation. Remove conjugate.
8.	 Wash 3 times for 5 minutes with 1.8 ml wash buffer by agitation. Remove wash buffer after every washing step.



SUBSTRATE

9.		Pipette 1050 µl dist. H ₂ O and 450 µl substrate into each incubation tray with strip.
10.		Incubate for 15 minutes at 20 - 32 °C/68 - 89.6 °F with agitation, protected from intense light. Remove substrate.
STOP		
11.		Pipette 2 ml dist. H ₂ O into each incubation tray with strip. Incubate 1 minute with agitation. Remove dist. H ₂ O. Repeat this step one time.
12.	Immediately transfer wet strips to scanner.	

AESKUBLOTS® Food Intolerance are also intended to be performed on the HELIA® Automated Blot System.

Reagent preparation for HELIA® Automated Blot System: Dilute 1 part wash buffer concentrate (WASH) with 19 parts ultrapure water (e.g. 50 mL wash buffer concentrate and 950 mL ultrapure water, respectively 100 mL wash buffer concentrate and 1900 mL ultrapure water) to obtain a ready-to-use wash buffer. All other reagents are ready to use when processed in HELIA® Automated Blot System.

For detailed handling of the test on HELIA® Automated Blot System refer to the instruction manual of the HELIA® Automated Blot System.

8 Quantitative Interpretation

8.1 Analysis

The analysis of the strips is carried out by means of using AESKU.SCAN software version 3.0 or higher. Please also refer to the instructions for use of AESKU.SCAN.

Visual interpretation of test results is not recommended.

Test results can be considered valid, if:

- Functional controls are visible
- Standards are visible with increasing intensity from standard 1 to 3
- Negative control is < standard 1

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

Evaluate strips according to the instructions for use of AESKU.SCAN software.

Quantitative result analysis is carried out by means of comparing the colour intensities of the individual foods / food mixtures with the colour intensity of the standards.

Using a HELIA® Automated Blot System, the results are analysed automatically. The results

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can be determined in kU_A/L and by class.

Correlation between Food Intolerance class and food-specific IgG₄ (Table 1)

Class	kU _A /L	Result
0	< 0.35	negative, no clinical significance
1	≥ 0.35 - < 0.7	low food-specific IgG ₄ concentration
2	≥ 0.7 - < 3.5	
3	≥ 3.5 - < 17.5	moderate food-specific IgG ₄ concentration
4	≥ 17.5 - < 50	
5	≥ 50 - < 100	high food-specific IgG ₄ concentration
6	≥ 100	

The following technical issues should as well be checked: expiry date of (prepared) reagents, storage conditions, pipettes, equipment, incubation conditions and washing methods.

If the samples tested show aberrant values or any kind of deviation or if the validation criteria are not met because of reasons outside the operator's responsibility, please contact the manufacturer or the supplier of the test kit.

Medical laboratories might perform an in-house quality control by using their own controls and/or internal pooled sera, as stated in national regulations.

9 Technical Data

Sample material:	Serum, plasma
Sample volume:	75 µl of sample
Total incubation time:	107 minutes at 20 - 32°C/ 68 - 89.6 °F
Storage:	at 2 - 8 °C/ 35.6 - 46.4 °F; use original vials only.
Number of determinations:	24 tests (option 1) or 120 tests (option 2)



10 Performance Data

10.1 Relative Sensitivity and Specificity

Analytical specificity: Aeskublots® Food Intolerance detects food-specific IgG₄ antibodies. No significant influences on the test results could be observed with the interfering substances triglycerides, hemolysate, bilirubin, and IgE. No cross-reactivity with other immunoglobulin species is known.

Relative sensitivity/specificity: The test results have been evaluated in comparison to a CE-marked ELISA system for detection of food specific human IgG₄. The comparison has shown the following relative sensitivity/specificity values for the food extracts listed in Table 2 (threshold value 0.35 kUA/L): **f199** 90%/92%, **f17** 77%/100%, **f14** 80%/89%, **f49** 83%/69%, **f24** 100%/100%, **f89** 80%/100%, **f37** 67%/97%, **f300** 92%/85%, **f13** 88%/100%, **f3** 100%/97. There are no known limitations or interferences.

Lot to Lot precision: Assays with control sera were performed with three Lots. The Lot to Lot precision was 94%.

Inter-assay precision: Five assays with control serum were performed on five consecutive days to determine inter-assay precision (n = 24). The mean values were determined separately for each food, the results are summarized in Table 2. Index value differences between the assays of ≤ 1.5 are accepted for the inter-assay precision of all foods.

Inter-assay precision (Table 2)

Food code	Number of measurements	Mean (index value)	Inter assay index value difference (max vs. min)	Analysis
f199	24	6.0	0.1	Positive
f17	24	5.0	0.4	Positive
f14	24	3.6	0.6	Positive
f49	24	4.8	0.3	Positive
f24	24	0.4	0.2	Negative
f89	24	6.0	0.0	Positive
f37	24	0.7	0.2	Negative
f300	24	4.9	0.2	Positive
f13	24	4.5	0.5	Positive
f3	24	0.3	0.1	Negative

11 Disposal

The relevant precautions for the handling of human blood (blood components) and the safe disposal of items contaminated with blood must be observed. Please follow all local / international guidelines for handling biohazardous waste. The remaining components, if applicable, can be considered non-biological and disposed of accordingly.

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12 Literature

Hardman G und Hart G, "Dietary advice based on food-specific IgG results" Nutrition & Food Science 2007; 37(1): 16-23.

Müller U und Bayer W, „Zur Relevanz der Bestimmung von spezifischem IgE und spezifischem IgG/IgG4 in der Aufdeckung von Nahrungsmittelunverträglichkeiten“ Erfahrungsheilkunde 2007; 56:400-406.

David TJ, "Adverse reactions and intolerance to foods" Br Med Bull 2000; 56: 34-50.

Zar S, et al., "Food-specific IgG₄ antibody-guided exclusion diet improves symptoms and rectal compliance in irritable bowel syndrome" Scand J Gastroenterol, 2005; 40(7): 800-807.

Lothar Thomas, Labor und Diagnose. Indikation und Bewertung von Laborbefunden für die medizinische Diagnostik., 8. Auflage, TH Books.

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



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Product Desc.

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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	~ Catalogue 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	
	~ Conformità europea	~ EC Declaration of Conformity
	~ Déclaration CE de Conformité	~ Declaración CE de Conformidad
	~ Europäische Konformität	~ Ευρωπαϊκή συμφωνία
	~ Declaração CE de Conformidade	
	~ 24 determinazioni	~ 24 tests
	~ 24 tests	~ 24 pruebas
	~ 24 Bestimmungen	~ 24 προσδιορισμοί
	~ 24 Testes	
	~ 120 determinazioni	~ 120 tests
	~ 120 tests	~ 120 pruebas
	~ 120 Bestimmungen	~ 120 προσδιορισμοί
	~ 120 Testes	
	~ Rispettare le istruzioni per l'uso	~ See instructions for use
	~ Voir les instructions d'utilisation	~ Ver las instrucciones de uso
	~ Gebrauchsanweisung beachten	~ Λάβετε υπόψη τις οδηγίες χρήσης
	~ Ver as instruções de uso	
	~ Da utilizzarsi entro	~ Use by
	~ Utilise avant le	~ Utilizar antes de
	~ Verwendbar bis	~ Χρήση μέχρι
	~ Utilizar antes de	
	~ Conservare a 2 - 8 °C	~ Store at 2 - 8 °C (35.6 - 46.4 °F)
	~ Conserver à 2 - 8 °C	~ Conservar a 2 - 8 °C
	~ Lagerung bei 2 - 8 °C	~ Φυλάσσεται στους 2 - 8 °C
	~ Conservar entre 2 - 8 °C	
	~ Prodotto da	~ Manufactured by
	~ Fabriqué par	~ Fabricado por
	~ Hergestellt von	~ Κατασκευάζεται από
	~ Fabricado por	
STRIP	~ Strip di nitrocellulosa rivestita	~ Coated nitrocellulose strip
	~ Strip de nitrocellulose couché	~ Tira de nitrocelulosa recubierta
	~ Beschichtete Nitrocellulose-Streifen	~ Επίστρωση λωρίδα νιτροκυτταρίνης
	~ Tira de nitrocelulose revestido	
WASH 20x	~ Tampone di lavaggio	~ Wash buffer
	~ Tampon de Lavage	~ Solución de lavado
	~ Waschpuffer	~ Ρυθμιστικό διάλυμα πλύσης
	~ Solução de lavagem	
CONJ	~ Coniugato	~ Conjugate
	~ Conjugé	~ Conjugado
	~ Konjugat	~ Σύζευγμα
	~ Conjugado	
SUB	~ Tampone substrato	~ Substrate buffer
	~ Substrat	~ Tampón sustrato
	~ Substratpuffer	~ Ρυθμιστικό διάλυμα υποστρώματος
	~ Substrato	