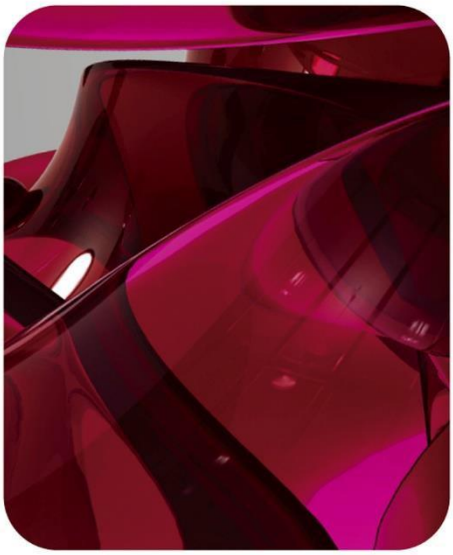
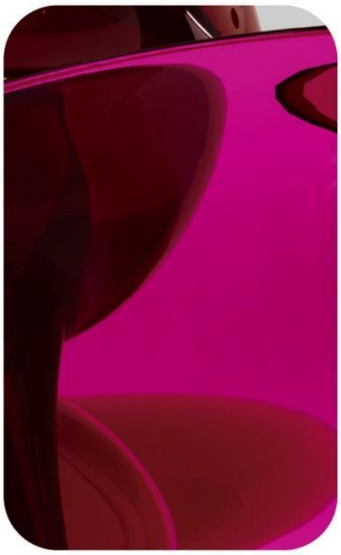
AESKU.DIAGNOSTICS
THE DIAGNOSTIC TOOL THAT WORKS


AESKUBLOTS®

THE DIAGNOSTIC TOOL THAT WORKS

INSTRUCTION MANUAL

AESKUBLOTS® Allergy

Ref 421001 + Ref 421001.BULK120, Ref 421002 + Ref 421002.BULK120, Ref 421003 + Ref 421003.BULK120, Ref 421004 + Ref 421004.BULK120, Ref 421005 + Ref 421005.BULK120, Ref 421006 + Ref 421006.BULK120, Ref 421407 + Ref 421407.BULK120, Ref 421408 + Ref 421408.BULK120, Ref 421009 + Ref 421009.BULK120, Ref 421010 + Ref 421010.BULK120, Ref 421411 + Ref 421411.BULK120, Ref 421412 + Ref 421412.BULK120, Ref 421413 + Ref 421413.BULK120

DIN EN ISO 13485

Instruction Manual

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1 Intended Use

AESKUBLOTS® Allergy is a membrane based enzyme immunoassay for quantitative detection of allergen-specific IgE antibodies against allergens/allergen mixtures and total IgE 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 a tool in diagnosis of type I allergy.

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

In diagnostic testing for allergies, 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. The higher the concentration of IgE, the greater the likelihood of a direct correlation with symptoms. To make a diagnosis, the test results must always be considered in conjunction with the symptoms and medical history.
Note! *AESKUBLOTS® Allergy* is used to test allergens 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 antigens are applied as lines on a nitrocellulose membrane. Membrane strips with specific antigens at exactly defined positions are incubated in serum or plasma samples diluted 1:10. Patient's antibodies, if present in the specimen, bind to the antigen. The unbound fraction is washed off in the following step. Afterwards, anti-human IgE immunoglobulins conjugated to Digoxigenin (conjugate 1) are incubated and react with the antigen-antibody complex of the samples. Unbound conjugate 1 is washed off in the following step. Afterwards, anti-Digoxigenin immunoglobulins conjugated to Horseradish peroxidase (conjugate 2) are incubated and react with the antigen-antibody-conjugate 1 complex of the samples. Unbound conjugate 2 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 1, IgE	1 x 15 ml	3 x 20 ml	yellow	colorless	anti-human immunoglobulin E (IgE) conjugated to Digoxigenin
Conjugate 2, Digoxigenin	1 x 15 ml	3 x 20 ml	green	colorless	anti-Digoxigenin conjugated to horseradish peroxidase
TMB Substrate	1 x 15 ml	3 x 20 ml	black	colorless	stabilized TMB/H_2O_2
Membrane strips	24 strips	120 strips	see certificate	N/A	coated antigens (see certificate)
Tweezers, adhesive strip double sided	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, digital 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 (USP 26 – NF 21) and the European Pharmacopeia (Eur.Ph. 4th ed.).					

4 Storage and Shelf Life

Store all reagents and membrane-strips at 2-8°C/35-46°F in their original containers. Once prepared, reconstituted solutions are stable at 2-8°C/35-46°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

Although this product is not considered particularly toxic or dangerous under the conditions of intended use, refer to the following for maximum safety:

Recommendations and precautions

The reagents contain the following substances below the concentration limits: the wash buffer and the antibody solution 1 contain ProClin (sensitizing), the TMB substrate contains 3,3',5,5'-tetramethylbenzidine and *N*-methyl-2-pyrrolidone (toxic to reproduction). The antibody solutions contain the preservative chloracetamide (toxic to reproduction and sensitising). Avoid contact with skin! Wear suitable protective equipment! For more information see Material Safety Data Sheet (available on request). Do not smoke, eat or drink when manipulating the kit! Do not pipette by mouth! Handle patient samples as if capable of transmitting infectious diseases and according to national requirements.

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® Allergy* 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 red line stands for *AESKUBLOTS® Allergy.* The lower line specifies the test. The complete color code and the allergen composition of each test is 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, Food Intolerance 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 and mix well 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 allergen-specific IgE, 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 allergens 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 allergens. Sensitisation to other allergens not contained in the *AESKUBLOTS® Allergy* test cannot be ruled out. Patients with clinical symptoms and Allergy class 0 reactions to the respective allergens/allergen mixtures should be referred to a specialist for further investigation.

The binding capacities of allergen-specific IgE antibodies can vary from allergen to allergen. Therefore, identical classes for various allergens will not necessarily correspond to the same sIgE 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® Allergy* do not necessarily correlate with specific IgE antibody titers obtained by other methodologies.

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/35-46°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

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).

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, 9 and 12.

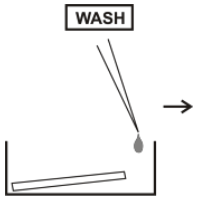
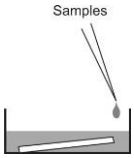
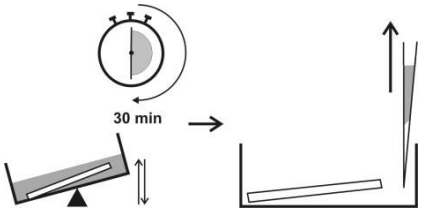
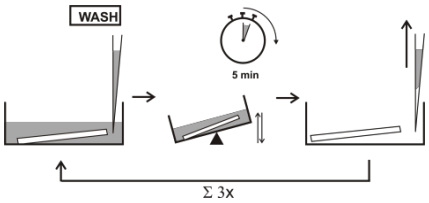
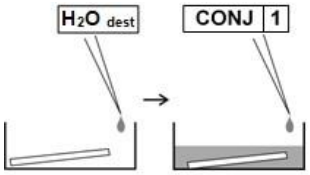
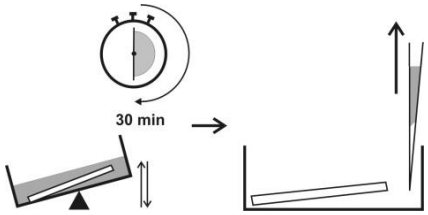
Do not let strip dry out during incubation steps.

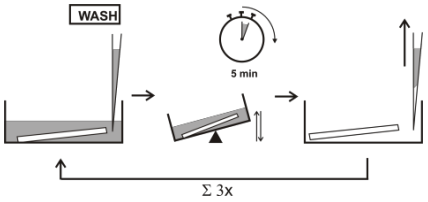
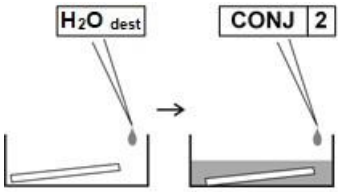
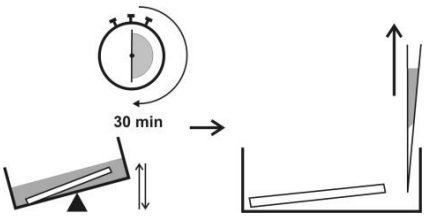
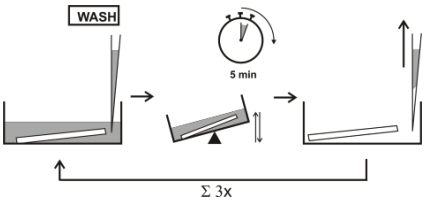
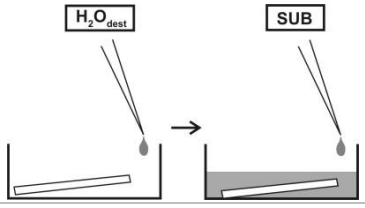
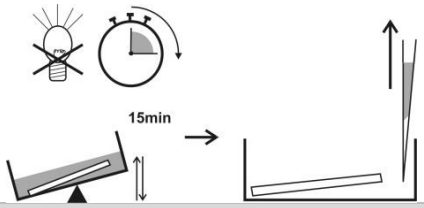
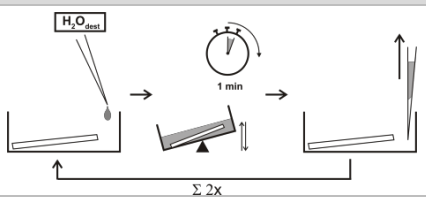
Do not touch strip with fingers, use tweezers.

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

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

Give sample buffer, conjugate and substrate together with the wash buffer to one side of 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 1350 µl wash buffer in the incubation tray.
CONTROLS & SAMPLES	
3.	 Pipette 150 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 1 + 2	
6.	 Pipette 1050 µl dist. H_2O and 450 µl conjugate 1 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.
9.		Pipette 1050 µl dist. H_2O and 450 µl conjugate 2 into each incubation tray with strip.
10.		Incubate for 30 minutes at 20-32 °C/68-89.6 °F with agitation. Remove conjugate.
11.		Wash 3 times for 5 minutes with 1.8 ml wash buffer by agitation. Remove wash buffer after every washing step.
SUBSTRATE		
12.		Pipette 1050 µl dist. H_2O and 450 µl substrate into each incubation tray with strip.
13.		Incubate for 15 minutes at 20-32 °C/68-89.6 °F with agitation, protected from intense light. Remove substrate.
STOP		
14.		Pipette 2 ml dist. H_2O into each incubation tray with strip. Incubate 1 minute with agitation. Remove dist. H_2O. Repeat this step one time.
15.	Immediately transfer wet strips to scanner.	

AESKUBLOTS® Allergy are also intended to be performed on the HELIA® line immunoassay analyzer.

Reagent preparation for HELIA: 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®. For detailed handling of the test on HELIA® refer to the instruction manual of the HELIA®.

8 Result 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 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 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 allergens / allergen mixtures with the colour intensity of the standards.

Using a HELIA® line immuno assay analyzer, the results are analysed automatically.
The results can be determined in kU/L and by class.

Correlation between Allergy class and specific IgE (Table 1)

Class	kU/L	Result
0	< 0.35	negative, no clinical significance
1	≥ 0.35 - < 0.7	low allergen-specific IgE concentration,
2	≥ 0.7 - < 3.5	of partial clinical significance
3	≥ 3.5 - < 17.5	moderate allergen-specific IgE concentration,
4	≥17.5 - < 50	often accompanied by clinical symptoms
5	≥ 50 - < 100	high allergen-specific IgE concentration,
6	≥ 100	clinical symptoms in most cases

Disposal: The precautions for handling human blood (blood components) and safely disposing of items contaminated with blood must be observed. Please heed all local/international guidelines on the handling of biohazardous waste. The remaining components can be regarded, where applicable, as non-biologic and disposed of accordingly.

To measure total IgE a different qualitative classification applies: class 0 – 2 <100kU/L (result seldom clinically relevant), class 3 – 6: ≥ 100 kU/L (result often clinically relevant).

Note: a negative result for total IgE does not preclude a positive result for allergen specific IgE.

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:	150 µl of sample
Total incubation time:	152 minutes at 20-32°C/68-89.6°F
Storage:	at 2-8°C/35-46°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® Allergy* detects allergen-specific IgE antibodies. No significant influences on the test results could be observed with the interfering substances triglycerides, hemoglobin and bilirubin. Increased IgG contents (>2200 mg/dL) caused by illness might decrease the specificity of the test results. Examples of diseases with increased IgG contents are acute or chronic infections, cirrhosis of the liver, hepatitis, multiple sclerosis, multiple myeloma, autoimmune diseases.

Relative sensitivity/specificity: The test results have been evaluated in comparison with the Thermo Fisher Scientific ImmunoCAP® system. The comparison has shown the following relative sensitivity/specificity values for the allergens listed in Table 2 (threshold value 0.35 kU/L): **e1** 92%/92%, **dx2** 100%/75%, **tx21** 83%/75%, **w1** 75%/83%, **w6** 92%/83%, **f1** 63%/94%, **f4** 83%/75%, **f14** 82%/85%, **f24** 80%/86%, **f25** 92%/83%. Indirect traceability to WHO standard 75/502 for IgE has been demonstrated by the above comparison. There are no known limitations or interferences.

Lot to Lot precision: Assay with control sera were performed with three Lots. The Lot to Lot precision was >99%.

Inter-assay precision: Five assays with control serum were performed on five days to determine inter-assay precision (n = 24). The mean values were determined separately for each allergen. 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 allergens.

Inter-assay precision (Table 2)

Allergen code	Number of measurements	Mean (index value)	Inter assay index value difference (max vs. min)	Analysis
e1	24	3.7	0.8	Positive
dx2	24	4.5	0.6	Positive
tx21	24	3.6	0.5	Positive
w1	24	3.1	0.7	Positive
w6	24	3.5	0.6	Positive
f1	24	0.5	0.1	Negative
f4	24	2.8	0.7	Positive
f14	24	3.0	0.6	Positive
f24	24	0.9	0.2	Negative
f25	24	4.9	0.7	Positive

11 Literature

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Ahlstedt S. Understanding the usefulness of specific IgE blood tests in allergy. Clin Exp Allergy 2002;32:11-6. 3. Martinez FD, et al. Asthma and wheezing in the first six years of life. N Engl J Med 1995;332:133-8.

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Gustafsson D, et al. In vitro diagnosis of atopic allergy in children. A comparison between total IgE, conventional RAST and a new multi RAST (Phadiatop). Allergy 1988 Feb;43:105-8.

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Chang ML, Cui C, Liu YH, Pei LC, Shao B. Analysis of total immunoglobulin E and specific immunoglobulin E of 3721 patients with allergic disease. Biomed Rep 2015;3:573-577.

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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

12 History

Product reference numbers added on front page, update of amount of reagent bottles and corresponding filled volume, Lot to Lot data added, note for disposal specification added.

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	
CE	¨ Conformità europea	¨ EC Declaration of Conformity
	¨ Déclaration CE de Conformité	¨ Declaración CE de Conformidad
	¨ Europäische Konformität	¨ Ευρωπαϊκή συμφωνία
	¨ Déclaracão CE de Conformidade	
Σ 24	¨ 24 determinazioni	¨ 24 tests
	¨ 24 tests	¨ 24 pruebas
	¨ 24 Bestimmungen	¨ 24 προσδιορισμοί
	¨ 24 Testes	
Σ 120	¨ 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 instrucões de uso	
	¨ 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	¨ Store at 2-8°C (35-46°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 nitrocelluslosa rivestita	¨ Coated nitrocellulose strip
	¨ Strip de nitrocellulose couché	¨ Tira de nitrocelulosa recubierta
	¨ Beschichtete Nitrozellulose-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
	¨ Solucão de lavagem	
CONJ 1	¨ Coniugato 1	¨ Conjugate 1
	¨ Conjugé 1	¨ Conjugado 1
	¨ Konjugat 1	¨ Σύζευγμα 1
	¨ Conjugado 1	
CONJ 2	¨ Coniugato 2	¨ Conjugate 2
	¨ Conjugé 2	¨ Conjugado 2
	¨ Konjugat 2	¨ Σύζευγμα 2
	¨ Conjugado 2	
SUB	¨ Tampone substrato	¨ Substrate buffer
	¨ Substrat	¨ Tampón sustrato
	¨ Substratpuffer	¨ Ρυθμιστικό διάλυμα υποστρώματος
	¨ Substrato	
	¨ Non riutilizzare	¨ Do not reuse
	¨ Ne pas réutiliser	¨ No reutilizar
	¨ Nicht wiederverwenden	¨ Μην επαναχρησιμοποιείτε
	¨ Não reutilizar	