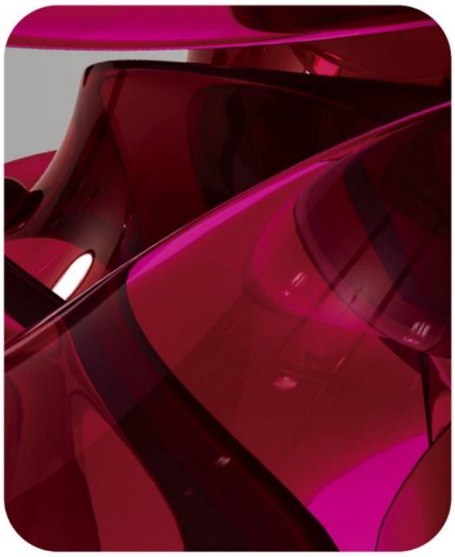
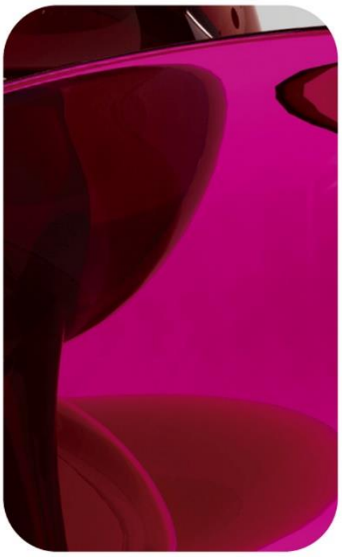
AESKU. DIAGNOSTICS
THE DIAGNOSTIC TOOL THAT WORKS


AESKUBLOTS®

THE DIAGNOSTIC TOOL THAT WORKS

INSTRUCTION MANUAL

AESKUBLOTS® Gastro Pro

Ref 4005

Instruction Manual

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1 Intended Use

***AESKUBLOTS*® Gastro Pro** is a membrane based enzyme immunoassay for the qualitative detection of IgG and IgA antibodies against gliadin, tTg neo-epitope, mannan (ASCA), parietal cell antigen and intrinsic factor in human serum. Antigens are located as parallel lines at exactly defined positions on a nitrocellulose membrane.

The assay is a tool in diagnosis of celiac disease, pernicious anaemia and inflammatory bowl diseases.

2 Clinical Application and Principle of the Test

Each section of the gastrointestinal tract may be affected by autoimmune gastrointestinal diseases. The diseases are often diagnosed years after the first onset of symptoms and in many cases they have a severe course.

Celiac patients often have an IgA deficiency. In order to avoid false-negative results, this test detects IgA and IgG antibodies.

Antibodies against:

- gliadin are typical for celiac disease. The IgA type is essentially specific for celiac disease. Antibodies of the IgG type occur in 40-50 % of patients with Crohn's disease and also in 10-20 % of patients with ulcerative colitis.
- the neoepitope of tTg (neo-tTg; tissue transglutaminase cross-linked to gliadin specific peptides) constitutes a reliable marker of celiac disease and dermatitis herpetiformis (Duhring's disease). Due to the structural similarity with the physiological epitopes, antibodies against neo-tTg exhibit greater sensitivity (98-100%) and specificity (93-96%) than anti-tTg antibodies.
- Mannan (ASCA) have a specificity of 97 % for Crohn's disease. They are essential for the differential diagnosis of Crohn's disease and ulcerative colitis. Up to 75 % of Crohn's disease patients show, in contrast to patients with ulcerative colitis, an increased antibody level.
- parietal cells can be detected by immunofluorescence test in 80-90 % of patients with pernicious anemia, but can also be found in 2-5 % of healthy individuals. They also occur in patients with autoimmune endocrinal diseases and chronic atrophic gastritis type A.
- intrinsic factor show for pernicious anaemia a sensitivity of 50-70 % with a specificity of 100 % in a population of healthy blood donors. Also, these antibodies are detected in patients with autoimmune thyroid diseases and chronic atrophic gastritis type A.

Principle of the test

The antigens are applied as lines on a nitrocellulose membrane. The membrane is blocked to prevent unspecific reactions. Membrane-strips with specific antigens at exactly defined positions are incubated in serum samples diluted 1:101. 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 immunoglobulins conjugated to horseradish peroxidase (conjugate) 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	Cap color	Solution color	Description / Contents
Blocking Reagent	3 x for 10 ml Concentrate each	white	N/A	Non-fat dry milk powder for preparation of 3 x 10 ml sample buffer
Wash Buffer (20x)	1 x 50 ml	white	colorless	20x concentrated for preparation of 1 L Tris buffer, pH 6.9 ± 0.2
READY TO USE				
Item	Quantity	Cap color	Solution color	Description / Contents
Conjugate, IgG	1 x 10 ml	blue	colorless	Anti-human immunoglobulin G (IgG) conjugated to horseradish peroxidase
Conjugate, IgA	1 x 10 ml	red	colorless	Anti-human immunoglobulin A (IgA) conjugated to horseradish peroxidase
TMB Substrate	1 x 10 ml	black	colorless	Stabilized TMB/H_2O_2
Membrane strips	24 strips	color coding: black	N/A	Coated antigens see Intended use
tweezers, reference template, scoring sheet, adhesive strip (double-sides, white)	1 pcs. each	N/A	N/A	N/A
incubation tray	3 pcs.	N/A	N/A	N/A
Labels for sample buffer	3 pcs.	N/A	N/A	N/A
MATERIALS REQUIRED, BUT NOT PROVIDED				
rocking platform, cylinder 1000 ml, pipette or cylinder for 10 ml, precision pipettes (10, 1000 µl), absorbent or filter 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 membrane-strips at 2-8°C/35-46°F in their original containers. Once prepared, reconstituted wash buffer as well as opened strips, conjugate and TMB are stable at 2-8°C/35-46°F for at least six weeks. Reconstituted blocking reagent is stable at 2-8°C/35-46°F for at least 3 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. 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

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

This product contains dilutions of human and/ or animal origin and should be considered as potentially infectious and should be handled according to national requirements. Do not smoke, eat or drink when manipulating the kit. Do not pipette by mouth.

Wash Buffer, 20x conc.

Hazardous ingredients according to regulation (EC) No. 1272/2008:

Name	EC-No.	CAS-No.	REACH registration No.	Amount (w/w)	Hazard class and category	Hazard statements
Reaction mass of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H -isothiazol-3-one (3:1)	911-418-6	55965-84-9	01-2120764691-48-xxxx	<0,0015%	Acute Tox. 2 Acute Tox. 2 Acute Tox. 3 Skin. Corr. 1C Eye Dam. 1 Skin Sens. 1A Aquatic Acute 1 Aquatic Chronic 1	H330 H310 H301 H314 H318 H317 H400 H410

Anti-Human IgA / IgA + IgG / IgG Conjugate

Hazardous ingredients according to regulation (EC) No. 1272/2008:

Reaction mass of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Methyl-2H -isothiazol-3-one (3:1)	911-418-6	55965-84-9	01-2120764691-48-xxxx	<0,01%	Acute Tox. 2 Acute Tox. 2 Acute Tox. 3 Skin. Corr. 1C Eye Dam. 1 Skin Sens. 1A Aquatic Acute 1 Aquatic Chronic 1	H330 H310 H301 H314 H318 H317 H400 H410
Phenol	203-632-7	108-95-2	01-2119882293-32-xxxx	<0,01%	Acute Tox 3 Acute Tox 3	H301 H311

					Acute Tox 3 Skin Corr. 1B Eye Dam. 1 Muta. 2 STOT RE 2 Aquatic Chronic 2	H331 H314 H318 H341 H373 H411
Substrate <u>Hazardous ingredients according to regulation (EC) No. 1272/2008:</u>						
Citric acid	201-069-1	77-92-9	01-2119457026-42-xxxx	1 - < 5 %	Eye Irrit. 2	H319
N-Methyl-2-pyrrolidon	212-828-1	872-50-4	-	0,1 - < 0,3 %	Repr. 1B Skin Irrit. 2 Eye Irrit. 2 STOT SE 3	H360D H315 H319 H335

Precaution phrases: P280: Wear protective gloves/protective clothing/eye protection/face protection.

P333 + P313: If skin irritation or a rash occurs: Get medical advice/attention.

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.

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

To differentiate between the various ***AESKUBLOTS***®-tests available, a color coding is applied above the reference line of the strips:

Color coding	***AESKUBLOTS***®
red	ANA-17 comp
orange	ANA-17 Pro
blue	Myositis Pro
brown	Liver Pro
purple	Vasculitis Pro
black	Gastro Pro
green	Borrelia-G and Borrelia-M

In case that the product information, including the labeling, is incorrect please contact the manufacturer or the supplier of the test kit.

Blocking Reagent and 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. Don't exchange substances between autoimmunity and borrelia diagnostic tests!

For handling of conjugate do not use polystyrene vessels.

Allow all components to reach room temperature (20-32°C/68-89°F) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test.

Never expose components to higher temperature than 37°C/ 98°F.

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

The intensity of the band colour does not necessarily correlate with antibody titers obtained by other reference methodologies.

Samples from apparent normal blood donors may contain autoantibodies.

If the patient sample contains elevated levels of immune complexes or other immunoglobulin aggregates, false positive results by non-specific binding cannot be ruled out.

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. Alternatively, the samples should be stored in tightly closed vials at 2-8°C/35-46°F for up to 48 h, or frozen at -20°C/-4°F for longer periods. Avoid repeated thawing and freezing. Do not use heat inactivated samples.

7 Assay Procedure

7.1 Preparations prior to starting

Confirm that no salt crystals have been formed in the concentrate. If this happened, dissolve the crystals by slightly warming, room temperature should be enough, the concentrate.

Dilute concentrated wash buffer 1:20 with distilled water (e.g. 950 ml plus 50 ml).

For preparation of sample buffer: add 10 ml wash buffer to one bottle Blocking Reagent and mix well.

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 step 2, 6, 9.

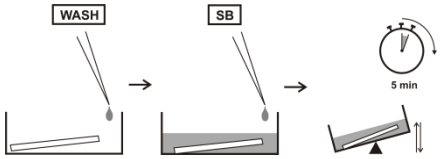
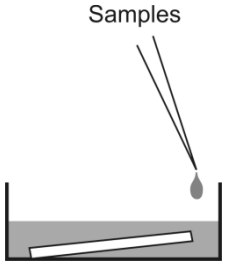
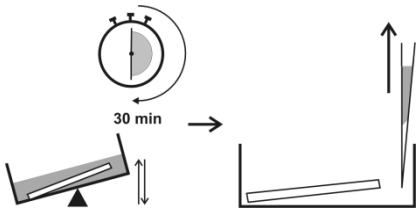
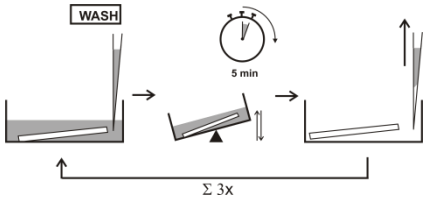
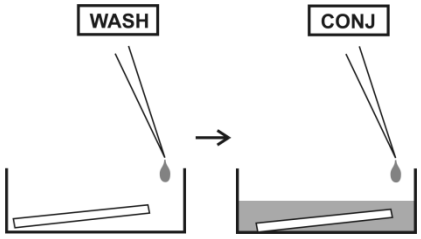
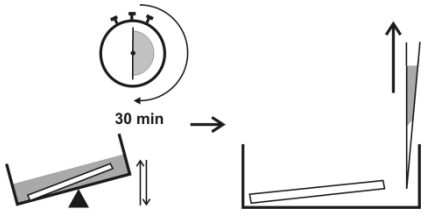
Do not let strip dry out during incubation steps.

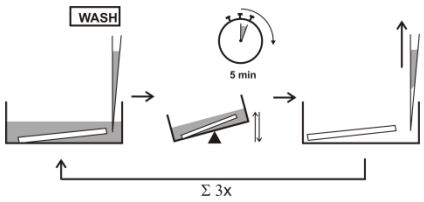
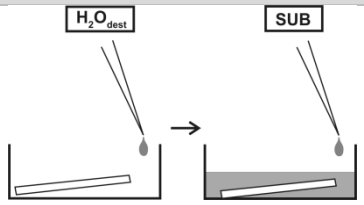
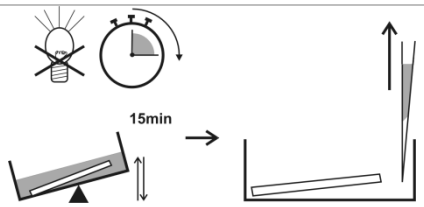
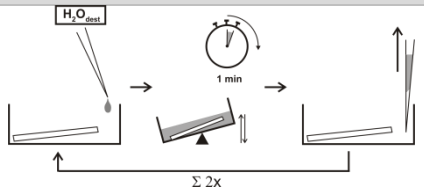
Do not touch strip with fingers, use tweezers.

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

Continuously shake strip during incubation steps.

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 colour coding upwards). Put 700 µl wash buffer and 300 µl sample buffer in the incubation tray. Moisten strip with the solution and incubate for 5 minutes with agitation.
CONTROLS & SAMPLES	
3.	 Pipette 10 µl serum sample into the designated incubation trays with sample buffer.
4.	 Incubate for 30 minutes at 20-32°C/68-89°F with agitation. After that remove sample completely.
5.	 Wash 3 times for 5 minutes with 1.5 ml wash buffer by agitation. Remove wash buffer after every washing step.
CONJUGATE	
6.	 Pipette 700 µl wash buffer and 300 µl conjugate into each incubation tray with strip.
7.	 Incubate for 30 minutes at 20-32°C/68-89°F with agitation. Remove conjugate.

8.		Wash 3 times for 5 minutes with 1.5 ml wash buffer by agitation. Remove wash buffer after every washing step.
SUBSTRATE		
9.		Pipette 700 µl dH_2O and 300 µl substrate into each incubation tray with strip.
10.		Incubate for 15 minutes at 20-32°C/68-89°F with agitation, protected from intense light. Remove substrate.
STOP		
11.		Pipette 2 ml dH_2O into each incubation tray with strip. Incubate 1 minute with agitation. Remove dH_2O. Repeat this step one time.
12.	Remove strip of the incubation tray. Dry strip between filter paper	
13.	Analyze results within 24 h.	

AESKUBLOTS® Gastro Pro is also intended to be automatically processed and evaluated on the HELIA® Automated blot system.
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) 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 Qualitative Interpretation

8.1 Manual Analysis

Test results can be considered valid, if:

- Functional control is visible
- Cut-off control is visible
- Color intensity of cut-off control is weaker than color intensity of functional control

Fix dried strip onto scoring sheet aligned with reference line. Align reference template with the strip reference line. Interpret results only in reference to cut-off control of each strip.

Each test kit contains a color copy with all bands provable in the test.

The analysis is carried out by means of comparing the color intensities of the bands with color intensity of the cut-off control. The test is equivocal if the intensities do not significant differ. Is the color more intensive the test result is positive, if the color intensity is weaker, the test is negative.

The results can be recorded on the scoring sheet.

In case that the values of the controls do not meet the criteria, the test is invalid and has to be repeated. We recommend retesting samples that are borderline.

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.

8.2 Software-supported evaluation

The analysis of the strips can be carried out by means of using AESKU.SCAN Software. Please refer to the instructions for use of AESKU.SCAN.

Test results can be considered valid, if:

- Functional control is visible
- Cut-off control is visible
- Color intensity of cut-off control is weaker than color intensity of functional control

<u>AESKU.SCAN 2.0:</u>

Fix dried strip onto scoring sheet (printable) aligned with reference line. Align reference template with the strip reference line.

Evaluate strips according to the instructions for use of AESKU.SCAN 2.0 software.

Qualitative result analysis is carried out by means of comparing the color intensities of the individual antigens with the color intensity of the cut-off control.

AESKU.SCAN 3.0:

Put strips within the incubation tray into the reader.

Evaluate strips according to the instructions for use of AESKU.SCAN 3.0 software.

Qualitative result analysis is carried out by means of comparing the color intensities of the individual antigens with the color intensity of the cut-off control.

HELIA®:

Using a HELIA® Automated blot system, the results are analyzed automatically. The results can be determined in Index-values.

The following interpretation according to the signal intensity is suggested:

Result Interpretation	Symbol	Index	Color
Negative	-	0.0 - <0.8	Colorless
Equivocal	+/-	≥0.8 - <1.15	Blue
Weak positive	+	≥1.15 - <2.5	Yellow
Positive	++	≥2.5 - <4.0	Red
Strong positive	+++	≥ 4.0	Dark red

In case that the values of the controls do not meet the criteria, the test is invalid and has to be repeated. We recommend retesting samples that are borderline.

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
Sample volume:	10 µl of sample
Total incubation time:	112 minutes at 20-32°C/68-89°F
Storage:	at 2-8°C/35-46°F; use original vials only.
Number of determinations:	24 tests

10 Performance Data

Relative Sensitivity and Specificity

In order to determine the positive agreement (relative sensitivity), 30 sera from IIF or ELISA antibody-positive patients were tested in ***AESKUBLOTS*®** **Gastro Pro.** For determination of the negative agreement (relative specificity), 100 sera from blood donors were analyzed.

	positive agreement (relative sensitivity)	negative agreement (relative specificity)
gliadin	100 %	100 %
Neo-tTg	100 %	100 %
mannan (ASCA)	100 %	100 %
parietal cell antigen	100 %	100 %
intrinsic factor	100 %	100 %

11 Literature

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Lerner A (2014). Serological Diagnosis of Celiac Disease –Moving Beyond the Tip of the Iceberg. International Journal of Celiac Disease. 2(2): 64-66.

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Holtmeier W, Caspary WF (1998). Antikörperdiagnostik bei Sprue/Zöliakie. Z. Gastroenterol. 36: 587**–**597.

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Oh R, Brown DL (2003). Vitamin B12 deficiency. Am Fam Physician. 67: 979**–**986.

Shan L, Molberg O, Parrot I, Hausch F, Filiz F, Gray GM, Sollid LM, Khosla C (2002). Structural basis for gluten intolerance in Celiac Sprue. Science 297:2275**–**2279.

Toh Ban-Hock, Alderuccio, F. (2004). Pernicious anaemia. Autoimmunity. 37: 357**–**361.

Toh Ban-Hock, van Driel Ian R. (1997). Pernicious anaemia. NEJM. 337:1441**–**1448.

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	
	¨ 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
	¨ Nitrozellulosemembran-Streifen mit aufgebrachten Antigenen	¨ 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	
Block-Reag	¨ Reagente bloccante	¨ Blocking Reagent
	¨ réactif de blocage	¨ Reactivo bloqueante
	¨ Blockier-Reagenz	¨ Αντιδραστήριο αποκλεισμού
	¨ Bloqueio de reagente	¨
RCNS 10ml	¨ Ricostituire con 10 mL	¨ Reconstitute with 10 mL
	¨ reconstituer avec 10 mL	¨ reconstituir con 10 mL
	¨ rekonstituiren mit 10 mL	¨ Ανασύσταση με 10 mL
	¨ reconstituir com 10 mL	¨
SB	¨ Tampone campione	¨ Sample buffer
	¨ Tampon Echantillons	¨ Tampón Muestras
	¨ Probenpuffer	¨ Ρυθμιστικό διάλυμα δειγμάτων
	¨ Diluente de amostra	
CONJ	¨ Coniugato	¨ Conjugate
	¨ Conjugé	¨ Conjugado
	¨ Konjugat	¨ Σύζευγμα
	¨ Conjugado	
SUB	¨ Tampone substrato	¨ Substrate buffer
	¨ Substrat	¨ Tampón sustrato
	¨ Substratpuffer	¨ Ρυθμιστικό διάλυμα υποστρώματος
	¨ Substrato	