AESKU.DIAGNOSTICS

THE DIAGNOSTIC TOOL THAT WORKS

AESKUSLIDES®

THE DIAGNOSTIC TOOL THAT WORKS

INSTRUCTION MANUAL

AESKUSLIDES® EMA

Ref 512.xxx

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INSTRUCTION MANUAL

EMA (Endomysium)

Standard Ref.	Description	Tests
512.050	**EMA IgA** (5 wells)	50
512.100	**EMA IgA** (10 wells)	100
512.060	**EMA IgG** (5 wells)	50
512.101	**EMA IgG** (10 wells)	100

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EMA (Endomysium)

1. INTENDED USE

***AESKUSLIDES*® EMA IgA and IgG** are an indirect immunofluorescence assays to detect autoantibodies against tissue transglutaminase (tTg) in human serum.
The test-kit is for professional use only in laboratories.

2. CLINICAL APPLICATION

Gluten-sensitive enteropathy or celiac disease is characterized by atrophy of the small intestinal villi leading to a so-called flat mucosa. It is caused by a pathological intolerance to gliadin, the alcohol-soluble fraction of gluten in wheat, rye and barley. As celiac disease is caused by the uptake of gluten, consequently a gluten-free diet cures the disease completely and thus has to be maintained for life-time. Renewed consumption of gliadin leads to a return of the symptoms. The disease is HLA-associated (>95 % of patients have DQ2 (DQA1*0501 and DQB1*0201)) and manifests at any age with a peak onset in early childhood, even in neonatals. The incidence rates range from 1 in 4000 to 1 in 300 in european countries.

Diagnosis of celiac disease is made by small intestinal biopsy (demonstrating flat mucosa) supported by serological markers. Antibodies against gliadin and anti-endomysium antibodies (EMA) are of major significance. They are detected so far by indirect immunofluorescence, which is restricted to subclass IgA only. The identification of tissue transglutaminase (tTg) as the major target antigen of EMA provided the opportunity of a more easy and reliable diagnosis of celiac disease. tTg is an enzyme that upon wounding is released from cells where it is thought to aid in tissue repair.

Anti-tTg antibodies show higher sensitivity and specificity than anti-Gliadin antibodies. Furthermore, they correlate tightly with the activity of the disease and thus are especially useful for diet monitoring. The determination of IgG antibodies to tTg is the only available specific serology for those 2-5 % of patients with IgA deficiency. A high number of subclinical cases have been detected by screening for anti-tTg, fostering the theory that the majority of celiac disease cases is undetected and untreated (Iceberg model).[1]

It is described that pemphigus patients show a specific pattern on monkey esophagus IIF.[2] Anti-skin antibodies are directed to the intercellular substance and are characteristic for pemphigus vulgaris, pemphigus foliaceus and paraneoplastic pemphigus, a distinction between the different pemphigus types is not possible with IIF.

[1] Matthias T et al.; Diagnostic Challenges in Celiac Disease and the Role of the Tissue Transglutaminase–Neo-Epitope. Clinic Rev Allerg Immunol. 2009; 298-301
[2] Bradwell A.R. et al.; Advanced Atlas of autoantibody patterns: Skin diseases; Birmingham: The Binding Site; 1999; 73-81

Antigen Characterization substrate: monkey esophagus

Cross - reactivity: Cross - reactivities are unknown

The detection of antibodies is based on the principle of indirect immunofluorescence assay (IIFA). Glass microscope slides are coated with tissue sections or cells (HEp-2 cells (ANA), Granulocytes (ANCA) or *Crithidia luciliae* (nDNA)). If the patient`s serum contains specific antibodies they will bind during the first incubation. After removing unbound material by washing steps, bound antibodies are detected by Fluorescein conjugated anti-human immunoglobulins during the second incubation. A specific green fluorescent staining of antigen-antibody-complex can be visualized with the aid of a fluorescent microscope.

3. KIT PROCEDURE

Please refer to Assay Procedure listed in Common Instructions, Section 11, for detailed instructions. The following details shall be used for the EMA kits:

- Counter staining time: 3-5 minutes
- Recommended Screening titer:
  - 1:5

4. INTERPRETATION

a. Interpretation EMA IgA

The endomysium is the supporting structure that surrounds the combination of smooth and striated muscle fibers that are found in the middle of the third of esophagus. It contains collagen and reticulin together with the endomysial target which has yet to be characterised.

Gliadin is the ethanol-soluble fraction of gluten which is the inflammatory antigen in coeliac disease. Antibodies are associated with coeliac disease and dermatitis herpetiformius.

b. Interpretation EMA IgG

The determination of IgG antibodies to tTg is the only available specific serology for those 2-5 % of patients with IgA deficiency.

Examples for dilution:

1:5	50 µL Serum	+	200 µL Sample Buffer
1:10	10 µL Serum	+	90 µL Sample Buffer
1:20	10 µL Serum	+	190 µL Sample Buffer
1:40	10 µL Serum	+	390 µL Sample Buffer
1:80	10 µL Serum	+	790 µL Sample Buffer

5. DATA INTERPRETATION SHEET

EMA

Date:	LOT.:
Slide No.:	Operator:

Well No.	ID	Dilution factor	F.I.	Endomysium Tunica muscularis mucosae	Smooth muscle	autoantibodies	remarks
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							

6. STANDARD KIT CONTENTS

		SLIDES				CONJUGATE			POSITIVE CONTROL		
Kit Ref.	**Kit Description**	**Ref.**	**Wells**	**Coated with**	**Quantity**	**Ref.**	**Description**	**Quantity**	**Ref.**	**Description**	**Quantity**
512.050	**EMA IgA** (5 wells)	**S512.050**	5	monkey Oesophagus	10 x	**C512.050**	**IgA** Capped blue: slightly blue coloured solution. Containing: BSA, Fluorescein (FITC) labelled Anti-human Antibody	1 x 3.5 ml	**PC512.050**	EMA positive control IgA Capped red: colourless solution. Containing: Human serum (diluted), sodium azide <0.1 % (preservative)	1 x 0.5 ml
512.100	**EMA IgA** (10 wells)	**S512.100**	10		10 x			2 x 3.5 ml			
512.060	**EMA IgG** (5 wells)	**S512.050**	5		10 x	**C512.060**	**IgG** Capped blue: slightly blue coloured solution. Containing: BSA, Fluorescein (FITC) labelled Anti-human Antibody	1 x 3.5 ml	**PC512.060**	EMA positive control IgG Capped red: colourless solution. Containing: Human serum (diluted), sodium azide <0.1 % (preservative)	1 x 0.5 ml
512.101	**EMA IgG** (10 wells)	**S512.100**	10		10 x			2 x 3.5 ml			

NOTE: The contents of the remaining components of the kits i.e. Common reagents (Neg. Ctrl, Mounting Medium etc.) are described below in section 7 COMMON REAGENTS CONTENTS.

7. COMMON REAGENTS CONTENTS

c. Common Reagents

Ref.	Reagent	Quantity / Volume		Description	Ready to use
NCIFA	Negative Control	1 x	0.5 ml	Capped green: colorless solution. Containing: Human serum (diluted), sodium azide <0.1 % (preservative)	YES
*** EBIFA**	Evans Blue 0.2 %	1 x	1.5 ml	Capped white: Blue colored solution Containing: PBS, Evans Blue. Dilute the Evans Blue 0.2 % 1:3000 in 1 x WBIFA	NO
**** MMIFA**	Mounting Medium	1 x	8 ml	Validated for use with the HELMED® Capped white: colorless solution Containing: PBS, Glycerin.	YES
***** MMIFA. Bulk**		1 x	12 ml		
WBIFA	Wash buffer (10 x)	1 x	100 ml	Capped white: colourless solution Dilute the concentrated buffer 1:10 in distilled water (e.g.: 100 ml + 900 ml). Containing: PBS, sodium azide (preservative).	NO
SBIFA	Sample buffer (1 x)	1 x	70 ml	Capped white: colorless solution for the dilution of patient sera Containing: BSA, PBS,sodium azide (preservative).	YES

Quantities are per kit. (*) must be ordered separately.
(**) for 512.050 and 512.060; (***) for 512.100 and 512.101

d. Materials required but not provided

1. Distilled water
2. Test tubes for sample dilution
3. Measuring flask
4. Volumetric pipette
5. Timer
6. Fluorescence microscope with FITC system, (490 nm excitation filter, 510 nm barrier filter)
7. Incubator tray
8. Staining dish
9. Pipetting tips
10. Cover slips (24 x 60 mm)
11. Squeeze wash bottle

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

8. STORAGE AND SHELF LIFE

Store all reagents at 2-8 °C / 35.6-46.4 °F, protected from intense light. The expiration date of each component is indicated on the respective label. Do not use reagents beyond the expiration date.

Store all reagents and the slides at 2-8 °C / 35.6-46.4 °F, in their original containers. Once prepared, reconstituted solutions are stable for at least 1 week at 2-8 °C / 35.6-46.4 °F. **Reagents and the slides shall be used within the expiry date indicated on each component, only.**

9. PRECAUTIONS OF USE

e. 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 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. All human source material used for some reagents of this kit (controls e.g.) has been tested by approved methods and found negative for HBsAg, Hepatitis C and HIV. However, no test can guarantee the absence of viral agents in such material completely. Thus handle kit controls and patient samples as if capable of transmitting infectious diseases and according to national requirements. The kit contains material of animal origin (BSA, Immunoglobulin) as stated in the table of contents, handle according to national requirements.

f. General directions for use

1. Do not pipette by mouth. Do not smoke, eat or drink when manipulating the kit.
2. Do not mix or substitute reagents from different lot numbers. This may lead to variations in the results.
3. Keep all flasks sealed after use to avoid bacterial contamination.
4. Always pipette all solutions with new sterile pipetting tips.
5. Never expose components to higher temperature than 37 °C / 98.6 °F (except the MMIFA).
6. Never let the slide wells dry out during the whole procedure.
7. Never freeze the slides.

Each laboratory should establish its own in-house controls upon its own techniques, controls, equipment and patient population according to its established procedures.

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.

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 proven: 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 a justified cause please contact our local representative.

10. SAMPLE COLLECTION, HANDLING AND STORAGE

Preparation of samples: use preferentially freshly collected serum samples. Blood withdrawal must follow national requirements. Collect blood samples aseptically.

Lipemic, icteric, hemolyzed or microbially contaminated specimens may cause interference.

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. Avoid repeated freezing and thawing.

11. ASSAY PROCEDURE

g. Preparation prior to pipetting

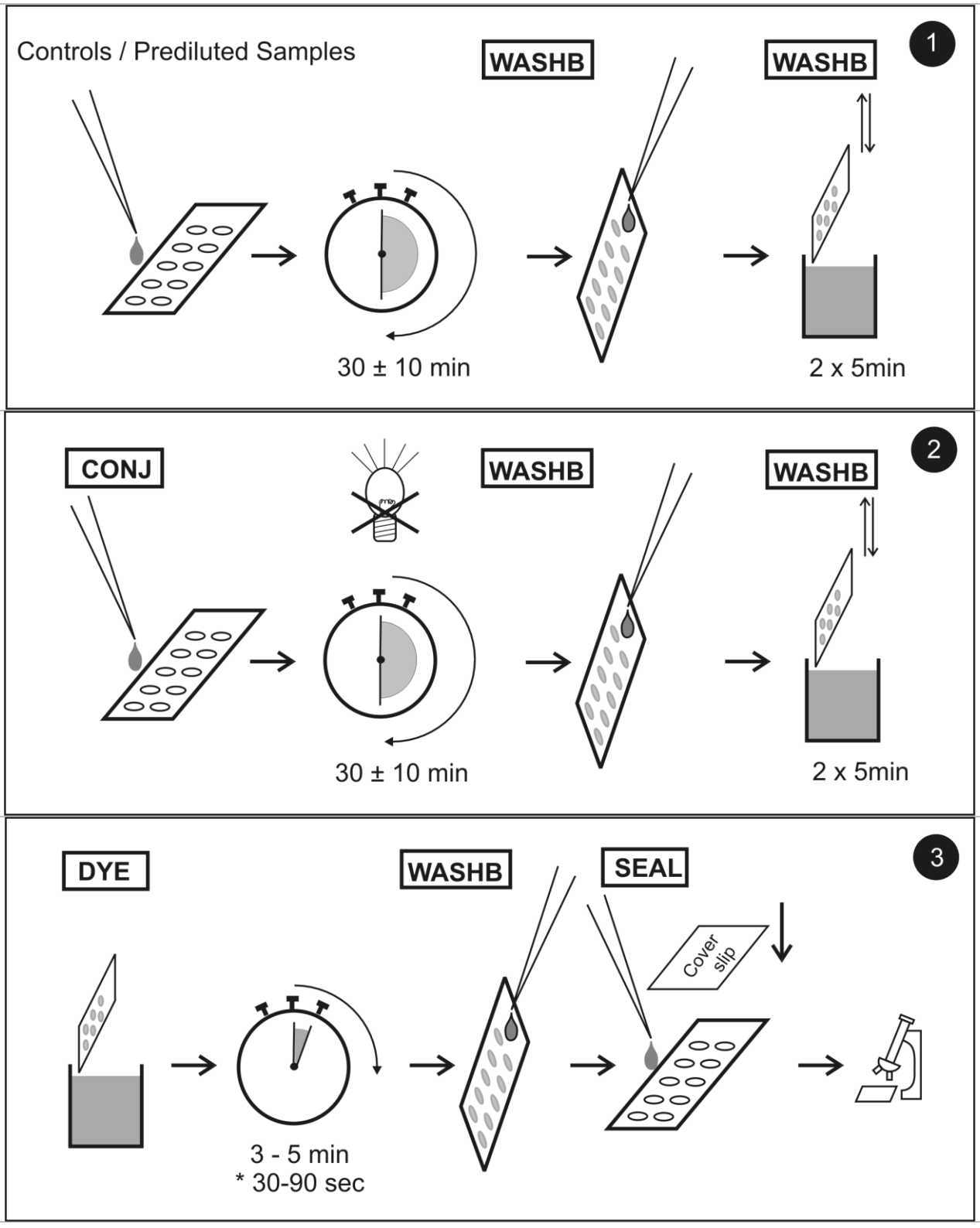
Allow all components to reach room temperature (20-26 °C / 68–78.8 °F) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test.

1. Preparation of the Wash Buffer: Dilute the concentrated buffer 1:10 with distilled water.
2. Dilution of samples: Dilute patient sera (for screening titer refer to ***Kit Procedure*** section above according to the product reference that you are using) with 1x Sample Buffer. These vary between HEp-2, nDNA, rLKS, EMA etc. kits.
3. Controls are ready to use.
4. Prepare a protocol: Data interpretation sheets are available in the ***Kit Procedure*** section according to the product reference that you are using.
5. In the case of increased viscosity and/or opacity, the Mounting Medium reagent (MMIFA) should be heated in a water bath for 15 minutes at 56 °C / 132.8 °F and then allowed to reach room temperature (20-26 °C / 68–78.8 °F) before use. After this procedure, the heated reagent can be used for up to 21 consecutive days, provided it is stored at 2-8°C / 35.6-46.4 °F, as specified on the reagent label and in the instructions for use.

h. Test Procedure

No.	Step description
1.	Remove required slide(s) from pouch(es) and mark them. Do not touch the wells. Do not allow the slides to dry out.
2.	**Preparation of incubator tray**: Place a small volume of deionized or distilled water in an incubator tray and place slide(s) on supports in the incubator tray. Incubate slide(s) 30 minutes ± 10 minutes at room temperature in the moist incubator tray. Use consistent incubation times for the conjugate. **First incubation**: Pipette an adequate volume of each diluted serum and controls (ready to use) into the appropriate wells, avoid direct contact of pipette with slide surface. Make sure that each well is completely covered with a corresponding serum. It is important to use as much test material as necessary to cover the well completely. But avoid a running between the wells because this may cause incorrect results.
3.	**Washing**: After incubation remove slides from incubator tray and rinse briefly with wash buffer using a squeeze wash bottle. Do not squirt buffer directly on the wells. NOTE: To prevent cross contamination tilt slide first towards one row and, carefully run a stream of wash buffer along the midline of the slide, allowing the wash buffer to run off the lower edge of the slide. Then tilt the slide towards the other row, and repeat this procedure, allowing the wash buffer to run off what is now the lower edge of the slide. Wash slide(s) 10 minutes with wash buffer in a slide staining dish. Avoid direct contact of solid items with the substrate. For optimal results change the buffer solution once after 5 minutes. Lift slide(s) out of staining dish and carefully remove excess washing buffer. NOTE: It is important that slide wells do not dry out during the procedure as this may lead to damage to the substrate. Please do not blot or dry the slide in any manner or allow slide to sit without fluorescent antibody reagent for longer than a few seconds.
4.	**Second incubation**: After the washing procedure return slide immediately to incubator tray and cover each well with an adequate volume of FITC-conjugate and make sure that the well is covered completely. Incubate slide(s) 30 minutes± 10 minutes at room temperature in the dark.
5.	**Washing**: After incubation remove slides from incubator tray and rinse briefly with wash buffer using a squeeze wash bottle. Do not squirt buffer directly on the wells. Wash slide(s) 10 minutes with wash buffer in a slide staining dish. For optimal results, change the buffer solution once after 5 minutes.
6.	***Optional counterstain**: Dilute counterstain (Evans Blue) 1:3000 in Wash buffer and mix well. Tilt counterstain into the staining dish and incubate the slides in it. Refer to ***Kit Procedure*** section above according to the product reference that you are using for incubation time details. Evans Blue covers unspecific background fluorescence. Remove slide(s) after the incubation time and rinse briefly with washing buffer. Remove excess washing buffer. Please do not blot or dry the slide in any manner.
7.	**Mounting Medium**: Add an adequate volume of mounting medium along midline of each slide. Carefully place coverslip in position, avoiding air bubbles.
8.	**Reading**: Read slide(s) immediately at 400 800 x total magnification with a fluorescent microscope. (490 nm excitation filter, 510 nm barrier filter).

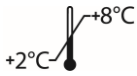
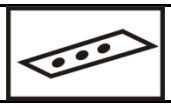
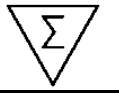
i. Workflow


Controls / Prediluted Samples
WASHB
WASHB
1
30 ± 10 min
2 x 5min
CONJ
WASHB
WASHB
2
30 ± 10 min
2 x 5min
DYE
WASHB
SEAL
3
Cover slip
3 - 5 min
* 30-90 sec

12. TROUBLESHOOTING

ERROR	POSSIBLE CAUSES	SOLUTION
Low cell density	• Cell lysis following prolonged contact with deionized water. • Buffer squirted directly on the substrate in the well.	Follow the recommended wash procedure.
	Proteolytic enzymes have attacked the substrate.	Inactivate serum.
Uneven fluorescence	Serum dried in the well, fluorescence stronger at the edge.	Always incubate in a humid environment.
	Serum does not cover the test well.	Apply an adequate volume of test material.
	Cross-reaction between the wells.	Avoid running between the wells in the first incubation.
	Marking the slide with a wax pencil produces a film on the slide.	Use a standard (non-wax) pencil.
	Microscope incorrectly adjusted.	Check the adjustment of the UV-lamp.
Diffuse picture	Slide incubated in refrigerator without cover.	Seal slide with nail polish or paraffin wax.
	I.F. Microscope is dirty. Possible scratches on the lens.	Clean the microscope according to its instructions.
Little or no fluorescence	Conjugate and slides thawed and refrozen.	Conjugate and slides stored at 2-8 °C / 35.6-46.4 °F.
	Controls diluted.	Check instructions, use ready to use kit controls.
	• Bacterial contamination of the sera or conjugate. • Microscope not adjusted. • pH-value of washing buffer too low (pH value 7.4 ± 0.2).	Check conditions.
	FITC conjugate exposed to light	Store conjugate protected from the light
Background fluorescence	• Incorrectly washed. • Slide dried out. • Lipemic, hemolytic sera. • Microscope error.	• Check the washing instructions. • Do not allow the slide to dry out. • Use only fresh sera. • Check correct filter / objective.

13. 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
	- Katalognummer	- 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
	- Declaração CE de conformidade	
	¨ 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	
	- 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	
DYE	- Colorante Blue-Evans	- Evans-Blue Dye
	- coloration au Bleu Evans	- Colorante Azul de Evans
	- Evans-Blue Färbelösung	- Evans Blue
	- Evans Blue	
CONTROL +	- Controllo positivo	- Positive Control
	- Contrôle Positif	- Control Positivo
	- Positiv Kontrolle	- Θετικός ορός ελέγχου
	- Controlo positivo	
CONTROL −	- Controllo negativo	- Negative Control
	- Contrôle Négatif	- Control Negativo
	- Negativ Kontrolle	- Αρνητικός ορός ελέγχου
	- Controlo negativo	
SEAL	- Mezzi di montaggio	- Mounting media
	- milieu de montage	- Medio de montaje
	- Mounting Medium	- Μέσο μονιμοποίησης
	- Meio de montagem	
CONJ	- Coniugato	- Conjugate
	- Conjugé	- Conjugado
	- Konjugat	- Σύζευγμα
	- Conjugado	
	- Vetrino per microscopio	- Microscope slide
	- lame de microscope	- Portaobjetos
	- Objektträger	- Αντικειμενοφόρο πλακίδιο
	- Lâmina	
WASHB 10x	- Tampone di lavaggio	- Wash Buffer
	- Tampon de Lavage	- Solución de lavado
	- Waschpuffer	- Ρυθμιστικό διάλυμα πλύσης
	- Solucão de lavagem	
SB 1x	- Tampone di campione	- Sample Buffer
	- Tampon de Echantillons	- Solución de muestras
	- Probenpuffer	- Ρυθμιστικό διάλυμα δειγμάτων
	- Solução para amostras	
	- Numero di determinazioni	- Number of determnations
	- Nombre de déterminations	- Número de determinaciones
	- Anzahl der Prüfungen	- Αριθμός προσδιορισμών
	- Número de determinações	