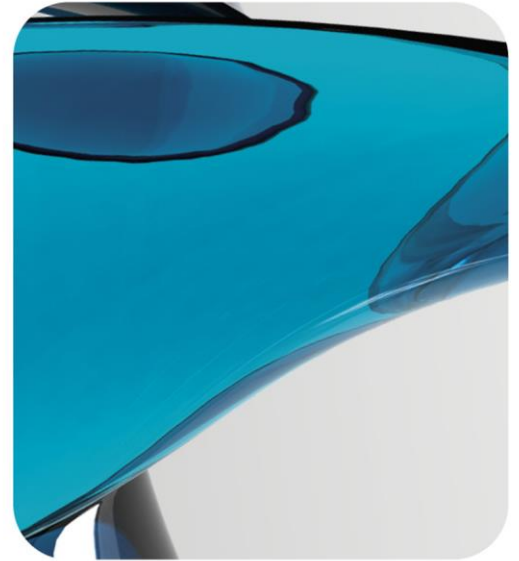
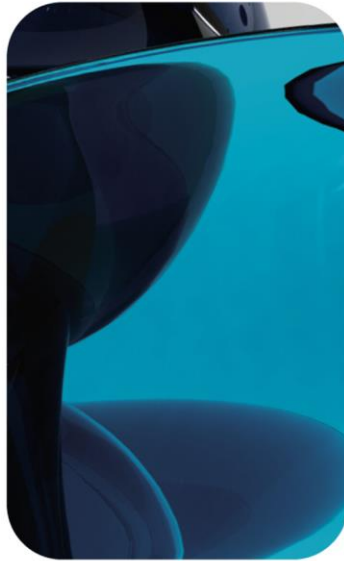
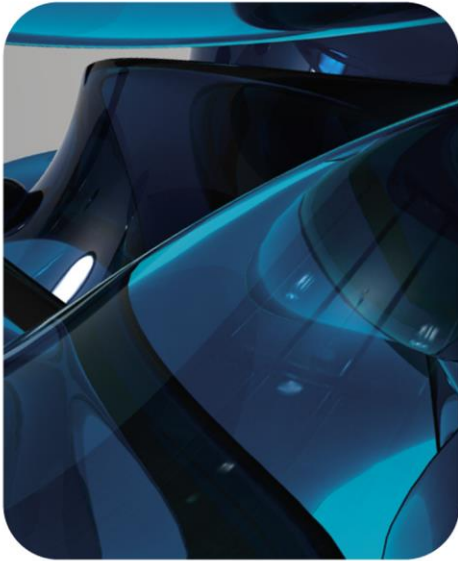




AESKU.DIAGNOSTICS
THE DIAGNOSTIC TOOL THAT WORKS



AESKUSLIDES[®]
THE DIAGNOSTIC TOOL THAT WORKS

**INSTRUCTION
MANUAL**

ENGLISH



AESKUSLIDES®
THE IFA PRODUCT LINE



INSTRUCTION MANUAL

nDNA (Crithidia Luciliae)

Standard Ref.	Description	Tests
53.100	nDNA (10 wells)	100



nDNA (*Crithidia luciliae*)

Ref.	Description	Tests
53.100	nDNA (10 wells)	100
53.100.Demo	nDNA (10 wells) Demo kit	20

1. INTENDED USE

AESKUSLIDES nDNA (*Crithidia luciliae*) is an indirect immunofluorescence assay to detect IgG antibodies to native double helix DNA in human serum.

2. CLINICAL APPLICATION

Antibodies binding to DNA belong to the group of anti-nuclear Antibodies (ANA) that have been observed in several autoimmune diseases. Antibodies reacting with native double-stranded (ds) DNA are regarded as being specific for systemic lupus erythematosus (SLE) and have been observed in approximately 50-80% of the patients. Antibodies against dsDNA are found during active phases of SLE. The amount of the serum concentration is positively correlated with the severity of the disease. Thus, detection of these autoantibodies is important for the diagnosis and the clinical monitoring of SLE. Consequently it has been established as 1 of 11 ACR-criteria of SLE. Most patients with SLE display IgG class antibodies against dsDNA. These autoantibodies are associated with lupus nephritis. Approximately 30% of the SLE patients develop IgA class anti-dsDNA antibodies, additionally. There have been suggestions that the presence of these IgA class anti-dsDNA antibodies may define a certain subset of SLE patients. Indeed studies demonstrated the association of this subclass with certain parameters of the disease activity, such as elevated erythrocyte sedimentation rate, or the consumption of complement component C3, as well as the clinical parameters of cutaneous vasculitis, acral necrosis and erythema. While no association was found for nephritis and arthritis.

IgM class anti-dsDNA antibodies were found in 52 % of the sera from patients with SLE. In contrast to IgG and IgA class autoantibodies, the subclass IgM antibodies do not correlate with disease activity. However, a highly significant negative correlation between IgM anti-dsDNA antibodies and lupus nephritis, including its laboratory parameters was demonstrated. Therefore IgM class anti-dsDNA antibodies may indicate a subset of lupus patients being protected against the risk of developing nephritis.

Antigen Characterisation: mitochondrial DNA from *Crithidia luciliae* (monoflagellate protozoa)

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 nDNA kits:

- Counter staining time: 30 to 90 seconds
- Recommended Screening titer: 1:10

4. INTERPRETATION

Screening titre 1:10

Crithidia luciliae contains a giant mitochondrion, also known as the kinetoplast which contains only dsDNA.

In the presence of antibodies against nDNA a homogenous fluorescence of the kinetoplast is visible or rather of the nucleus and the kinetoplast which is situated between the nucleus and the basal body near the flagellum.

The evaluation should always be performed with the positive and negative controls.

The sample must be assessed as nDNA negative if fluorescence of the basal body occur near the flagellum origin or the nucleus only and no specific nDNA antibodies exist.

Examples for dilution:

1:10	10µL Serum	+	90µL Samplebuffer
1:20	10µL Serum	+	190µL Samplebuffer
1:40	10µL Serum	+	390µL Samplebuffer
1:80	10µL Serum	+	790µL Samplebuffer

a.s.o.

Immunofluorescence shows a characteristic double spot pattern in the presence of anti-ds-DNA whilst only the nucleus is fluorescent with non-dsDNA nuclear antibodies. (dsDNA is an important autoantigene in SLE with a specificity of 95%).

In SLE patients antibodies to a variety of nuclear antigens may be observed. The strongest correlation to this disease is shown by Sm-antibody (glycoprotein) which appears as a speckled nuclear pattern in HEp-2 cells and nDNA-antibody (a peripheral or homogenous pattern in HEp-2 cells).

Antibodies to native double helix DNA are highly specific for SLE. Although low levels of nDNA antibody may be observed in other disease states e.g. Sjögren syndrome, Mixed Connective Tissue Disease (MCTD) and dermatomyositis, high titres of nDNA antibody are detected almost exclusively in SLE.¹

¹ Storch WB; Immunfluoreszenz-Fibel 2nd Edition; Blackwell Wissenschaftsverlag 1997

6. STANDARD KIT CONTENTS

6.1 STANDARD KITS

Kit Ref.	Kit Description	SLIDES (10x in each kit)			CONJUGATE (1x 4 ml)		POSITIVE CONTROL (1x 0.5ml)	
		Ref.	Wells	Coated with	Ref.	Description	Ref.	Description
53.100	nDNA (10 wells)	s53.100	10	<i>Crithidia Luciliae</i> cells	C53.100	IgG Capped blue: slightly blue coloured solution. Containing: BSA, Fluorescein (FITC) labelled Anti-human Antibody	PC53.100	nDNA positive control. Capped red: colourless solution. Containing: Human serum (diluted), sodium azide <0.1% (preservative)

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.

6.2 DEMO KITS

For the content of the demo kits refer to the corresponding certificate of analysis.

7. COMMON REAGENTS CONTENTS

a. Common Reagents

Ref.	Reagent	Quantity / Volume		Description	Ready to use
NCIFA	Negative Control	1x	0.5ml	Capped green: colourless solution. Containing: Human serum (diluted), sodium azide <0.1% (preservative)	YES
* EBIFA	Evans Blue 0.2%	1x	1.5ml	Capped white: Blue coloured solution Containing: PBS, Evans Blue. Dilute the Evans Blue 0.2% 1:3000 in 1x WBIFA	NO
MMIFA	Mounting Medium	1x	8ml	Validated for use with the HELMED® Capped white: colourless solution Containing: PBS, Glycerin.	YES
WBIFA	Washbuffer (10x)	1x	100ml	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	Samplebuffer (1x)	1x	70ml	Capped white: colourless solution for the dilution of patient sera Containing: BSA, PBS, sodium azide (preservative).	YES

Quantities are per kit. (*) must be ordered separately.

b. 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, (490nm excitation filter, 510nm barrier filter)
7. Incubator tray
8. Staining dish
9. Pipetting tips
10. Cover slips (24x60 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°C-8°C/35-46°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/35-46°F, in their original containers. Once prepared, reconstituted solutions are stable for at least 1 week at 2-8°C/35-46°F. **Reagents and the slides shall be used within the expiry date indicated on each component, only.**

9. PRECAUTIONS OF USE

a. 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.

b. 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.
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, hemolysed 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 8h, respectively stored tightly closed at 2-8°C/35-46°F up to 48h, or frozen at -20°C/-4°F for longer periods. Avoid repeated freezing and thawing.

11. ASSAY PROCEDURE

a. Preparation prior to pipetting

Allow all components to reach room temperature (20 - 26°C / 64 - 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 Samplebuffer. 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.



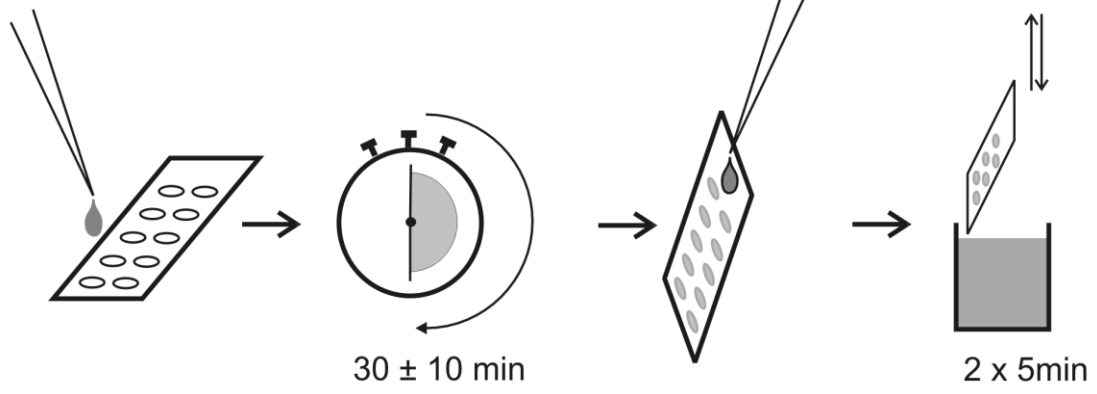
b. Test Procedure

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.	<p>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.</p> <p>Incubate slide(s) 30 minutes \pm 10 minutes at room temperature in the moist incubator tray. Use consistent incubation times for the conjugate.</p> <p>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.</p> <p>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.</p>
3.	<p>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.</p> <p>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.</p> <p>Lift slide(s) out of staining dish and carefully remove excess washing buffer.</p> <p>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.</p>
4.	<p>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.</p> <p>Incubate slide(s) 30 minutes \pm 10 minutes at room temperature in the dark.</p>
5.	<p>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.</p>
6.	<p>*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.</p> <p>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.</p>
7.	<p>Mounting Medium: Add an adequate volume of mounting medium along midline of each slide. Carefully place coverslip in position, avoiding air bubbles.</p>
8.	<p>Reading: Read slide(s) immediately at 400 - 800 x total magnification with a fluorescent microscope. (490 nm excitation filter, 510 nm barrier filter).</p>

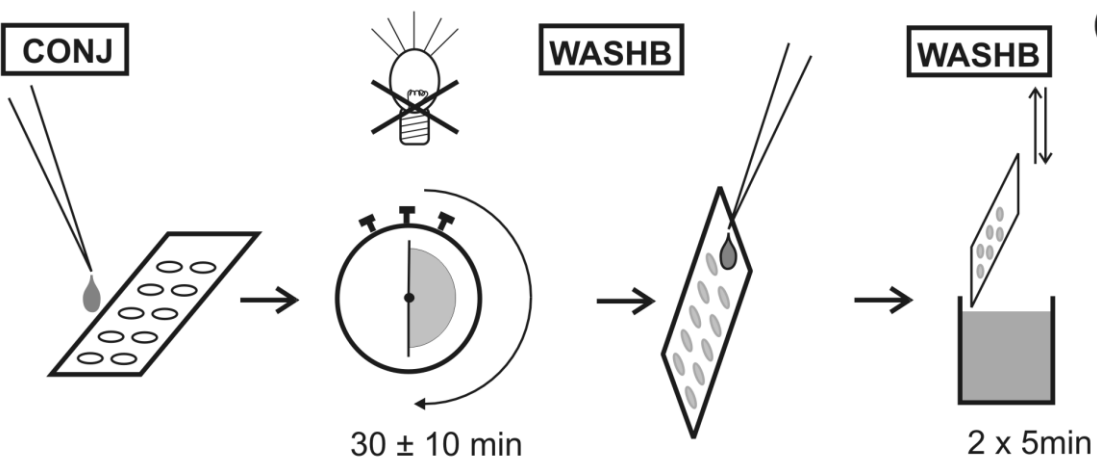


c. Work flow

Controls / Prediluted Samples



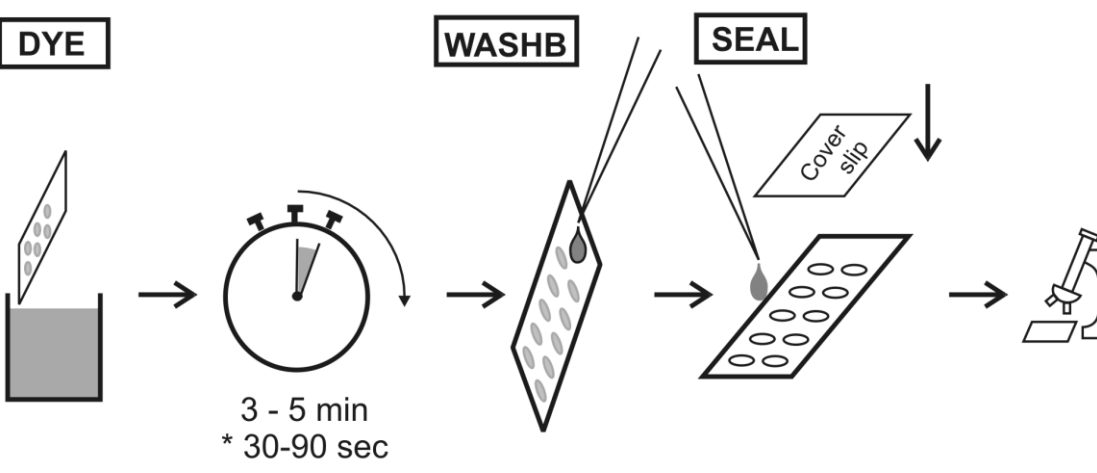
CONJ



DYE

WASHB

SEAL





12. TROUBLESHOOTING

ERROR	POSSIBLE CAUSES	SOLUTION
Low cell density	-Cell lysis following prolonged contact with deionised 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
Diffuse picture	Microscope incorrectly adjusted	Check the adjustment of the UV-lamp
	Slide incubated in refrigerator without cover	Seal slide with nail polish or paraffin wax
Little or no fluorescence	I.F. Microscope is dirty. Possible scratches on the lense	Clean the microscope according to its instructions
	Conjugate and slides thawed and refrozen	Conjugate and slides stored at 2°C-8°C/35-46°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
Background fluorescence	FITC conjugate exposed to light	Store conjugate protected from the light
	- 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



	- Diagnosi in vitro	ro diagnostic use
	- Pour diagnostic in vitro	- Para uso diagnóstico in vitro
	- In Vitro Diagnostikum	- In Vitro Διαγνωστικό μέσο
	- Para uso Diagnóstico in vitro	
	Numero d'ordine	Catalogue number
	Référence Catalogue	Numéro de catálogo
	Bestellnummer	Αριθμός παραγγελίας
	Número de catálogo	
	Descrizione lotto	Lot
	Lot	Lote
	Chargen Bezeichnung	Χαρακτηρισμός παρτίδας
	Lote	
	Conformità europea	EC Declaration of Conformity
	Déclaration CE de Conformité	Declaración CE de Conformidad
	Europäische Konformität	Ευρωπαϊκή συμφωνία
	Déclaracão 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 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 (35-46°F)
	Conservar 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	
	Colorante Blue-Evans	Evans-Blue Dye
	coloration au Bleu Evans	Colorante Azul de Evans
	Evans-Blue Färbelösung	Evans Blue
	Evans Blue	
	Controllo positivo	Positive Control
	Contrôle Positif	Control Positivo
	Positiv Kontrolle	Θετικός ορός ελέγχου
	Controllo positivo	
	Controllo negativo	Negative Control
	Contrôle Négatif	Control Negativo
	Negativ Kontrolle	Αρνητικός ορός ελέγχου
	Controllo negativo	
	Mezzi di montaggio	Mounting media
	milieu de montage	Medio de montaje
	Mounting Medium	Μέσο μονιμοποίησης
	Meio de montagem	
	Coniugato	Conjugate
	Conjugué	Conjugado
	Konjugat	Σύζευγμα
	Conjugado	
	Vetrino per microscopio	Microscope slide
	lame de microscope	Portaobjetos
	Objekträger	Αντικειμενοφόρο πλακίδιο
	Lamina	
	Tampone di lavaggio	Wash Buffer
	Tampon de Lavage	Solução de lavagem
	Waschpuffer	Ρυθμιστικό διάλυμα πλύσης
	Solución de lavado	
	Tampone di campione	Sample Buffer
	Tampon de Echantillons	Solución de Muestras
	Probenpuffer	Ρυθμιστικό διάλυμα δειγμάτων
	Solución de Muestras	
	XX determinazioni	XX tests
	XX tests	XX pruebas
	XX Bestimmungen	XX προσδιορισμοί
	XX Testes	