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# AESKUSLIDES®

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# INSTRUCTION MANUAL

**AESKUSLIDES® Rodent Tissues**

***Ref 517.xxx & 518.xxx***

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AESKUSLIDES®
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# INSTRUCTION MANUAL

## Rodent Tissues (rat/mouse LKS)

| Standard Ref. | Description | Tests |
|---|---|---|
| **517.050** | **rLKS** - rat, wrapped (5 wells) | 50 |
| **517.101** | **rLKS** - rat, wrapped (10 wells) | 100 |
| **517.051** | **rLKS** - rat, separated (5 wells) | 50 |
| **517.100** | **rLKS** - rat, separated (10 wells) | 100 |
| **518.050** | **mLKS** - mouse, separated (5 wells) | 50 |
| **518.100** | **mLKS** - mouse, separated (10 wells) | 100 |

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# Rodent Tissues (rat/mouse LKS)

## 1. INTENDED USE

The above ***AESKUSLIDES***® references are an indirect immunofluorescence assays to detect autoantibodies against for example mitochondrial (AMA), smooth muscle (ASMA), liver kidney microsomal (LKM) or circulating parietal cells (APCA) in human serum.

## 2. CLINICAL APPLICATION

Autoimmune diseases are caused by a disorder of the cellular and / or humoral immunological reaction. These reactions which normally occur against external influences may under certain circumstances turn against the body itself and thereby cause various diseases.

**ANA:** The presence of Anti-Nuclear-Antibodies maybe detected in every provided tissue by positive nuclear fluorescence. Besides this, it is not recommended to be used for screening of ANA patterns since the HEp-2 cells are must more sensitive and allows recognizing several different types of patterns.

**AMA:** Anti-mitochondrial antibodies (AMA) predominantly react with the inner membrane of the mitochondria (rich in phospholipids). AMA mostly appear with diseases such as primary biliary cirrhosis, pseudo - LE syndrome and various forms of chronic aggressive hepatitis. High AMA titer results are mainly found with non-suppurating gallbladder infections or primary biliary cirrhosis (positive results at about 90 %).

In these cases antibodies appear before the clinical symptoms and will hardly be influenced by therapy during the course of the disease.

Low antibody titers are observed with scleroderma, Sjögren syndrome, rheumatoid arthritis and other autoimmune diseases.

**ASMA:** Antibodies against smooth, unstriated muscle occur in various liver diseases, for example acute and chronic hepatitis, primary biliary cirrhosis, and other forms of liver cirrhosis. Furthermore, the detection of ASMA supports the diagnosis of SLE, infectious mononucleosis, breast and ovarian carcinoma and malignant melanomas.

**LKM:** Antibodies that bind to cytochrome p450 and are common associated to type 2 autoimmune hepatitis that predominantly occurs in a subgroup of girls and young women (80 % of prevalence). They can be also associated with hepatitis C.

**APCA:** Circulating antibodies against the structures of the parietal cell of the gastric mucosa are generally due to pernicious anemia. They may, however, also be detected with other diseases of the stomach (chronic atrophic gastritis, gastric ulcer), diseases of the thyroid (Hashimoto`s thyroiditis, myxedema) and more rarely with hypoferric anemia, diabetes mellitus and in older patients.

**Antigen Characterization substrate**: rat or mouse liver, kidney, stomach / rat or mouse kidney, stomach

**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 Rodent Tissue kits:

- Counter staining time: 3-5 minutes
- Recommended Screening titer: 1:20

## 4. INTERPRETATION

**R or M-LKS / R or M-KS:** The combined tissue section allows the differentiation of various antibodies within one test area and may thus be applied as a diagnostic test for the following autoimmune antibodies. (In case of diverse antibodies it is advisable to look for further diagnostic identification). The evaluation should always be performed with the positive and negative controls.

**ANA:** The presence of anti-nuclear-Antibodies maybe detected in every provided tissue by positive nuclear fluorescence.

**AMA:** The presence of anti-mitochondrial antibodies displays a fine granular cytoplasmatic fluorescence of the renal tubules. The distal tubules are richer in mitochondria and therefore display a more intense fluorescence in contrast to the proximal tubules.

**ASMA:** The presence of ASMA is indicated by a fluorescence of the smooth muscle fibres of the blood vessels of kidney and stomach, of muscularis mucosa, tunica muscularis ventriculi as well as the interglandular contractile fibrillae of the stomach mucosa.

**APCA:** Finely granular fluorescence of the parietal cells in the gastric mucous membrane indicates APCA. Since AMA also reacts with parietal cells, anti-mitochondrial antibodies (renal tubules) should be excluded in the APCA assessment.

**LKM:** A specific staining is seen in the cytoplasm of proximal renal tubules but not in distal. The liver shows homogenous staining of the hepatocytes and there is no staining seen in the stomach.

**AMA:**

- 1:20-1:80 (e.g. 10 µl Serum + 790 µl sample buffer) A positive reaction is found in several liver diseases
- >1:160 (e.g. 10 µl Serum + 1590 µl sample buffer) indicates biliary cirrhosis. AMA titers remain constant over a long period of time, and despite therapy so that the determination of titer as a measure of therapy control is not useful.

**ASMA:**

- 1:20-1:80 (e.g. 10 µl Serum + 790 µl sample buffer) A positive reaction is found in several liver diseases, viral hepatitis and primary biliary cirrhosis. However, the titers here may fall below the determination border. Low titers may be observed in patients with gallbladder infections, alcoholic cirrhosis, SLE and in 2 % of the normal, healthy population.
- >1:160 (e.g. 10 µl Serum + 1590 µl sample buffer) Chronic active hepatitis is indicated. In contrast to viral hepatitis the titers fall only slightly and may persist for several years. Patients with infectious mononucleosis may also show high ASMA titers.

**APCA:** The APCA titre provides no information about the disease state of the patient. The antibody determination should be evaluated together with the measurement of Intrinsic factor and / or histopathology results.

The appropriate end titer is that in which the patient serum shows a simple positive fluorescence. Weak fluorescence with titres between 1:20 and 1:40 or vagueness with respect to the clinical results should be checked by way of monitoring control. In such a case the samples should be collected about every 3 weeks and similarly tested.[1]

[1] Thomas L; Labor und Diagnose; 6th Edition; TH-Books GmbH

# 5. DATA INTERPRETATION SHEET

## LKS

| Date: | LOT: |
|---|---|
| Slide No.: | Operator: |

| Well No. | ID | Dilution factor | F.I. | Liver | Kidney | | Stomach | | Nucleus | Antibodies | Remarks |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Tubuli | Glomeruli | Parietalcells | Smooth muscle | | | |
| 1 | | | | | | | | | | | |
| 2 | | | | | | | | | | | |
| 3 | | | | | | | | | | | |
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| 17 | | | | | | | | | | | |
| 18 | | | | | | | | | | | |

# 6. STANDARD KIT CONTENTS

## 6.1. Standard Kits

| | | SLIDES | | | | CONJUGATE | | | POSITIVE CONTROL | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Kit Ref.** | **Kit Description** | **Ref.** | **Wells** | **Coated with** | **Quantity** | **Ref.** | **Description** | **Quantity** | **Ref.** | **Description** | **Quantity** |
| **517.050** | **rLKS** wrap. 5 well | S517.050 | 5 | Rat LKS tissues (L/K wrapped in stomach) | 10 x | CDTIFA | **IgG** Capped blue: slightly blue coloured solution. Containing: BSA, Fluorescein (FITC) labelled Anti-human Antibody | 1 x 3.5 ml | PCDTIFA | AMA positive control. Capped red: colourless solution. Containing: Human serum (diluted), sodium azide <0.1% (preservative) | 1 x 0.5 ml |
| **517.101** | **rLKS** wrap. 10 well | S517.101 | 10 | Rat LKS tissues (L/K wrapped in stomach) | 10 x | | | 2 x 3.5 ml | | | 1 x 0.5 ml |
| **517.051** | **rLKS** sep. 5 well | S517.051 | 5 | Rat LKS tissues (separated LKS sections) | 10 x | | | 1 x 3.5 ml | | | 1 x 0.5 ml |
| **517.100** | **rLKS** sep. 10 well | S517.100 | 10 | Rat LKS tissues (separated LKS sections) | 10 x | | | 2 x 3.5 ml | | | 1 x 0.5 ml |
| **518.050** | **mLKS** sep. 5 well | S518.050 | 5 | Mouse LKS tissues (separated LKS sections) | 10 x | | | 1 x 3.5 ml | | | 1 x 0.5 ml |
| **518.100** | **mLKS** sep. 10 well | S518.100 | 10 | Mouse LKS tissues (separated LKS sections) | 10 x | | | 2 x 3.5 ml | | | 1 x 0.5 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

## a. Common Reagents

| Ref. | Reagent | Quantity / Volume | | Description | Ready to use |
|---|---|---|---|---|---|
| **NCIFA** | Negative Control | 1x | 0.5 ml | Capped green: colorless solution. Containing: Human serum (diluted), sodium azide <0.1 % (preservative) | YES |
| *** EBIFA** | Evans Blue 0.2 % | 1x | 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 | 1x | 8 ml | Validated for use with the HELMED® Capped white: colorless solution Containing: PBS, Glycerin. | YES |
| ***** MMIFA. Bulk** | | 1x | 12 ml | | |
| **WBIFA** | Wash buffer (10 x) | 1x | 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) | 1x | 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 517.050, 517.051 and 518.050; (***) for 517.101, 517.100 and 518.100

## 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, (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

## c. 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.

## d. 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 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, 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 / 35.6-46.4 °F up to 2 weeks, or frozen at -20 °C / -4 °F for longer periods. Avoid repeated freezing and thawing.

# 11. ASSAY PROCEDURE

## e. Preparation prior to pipetting

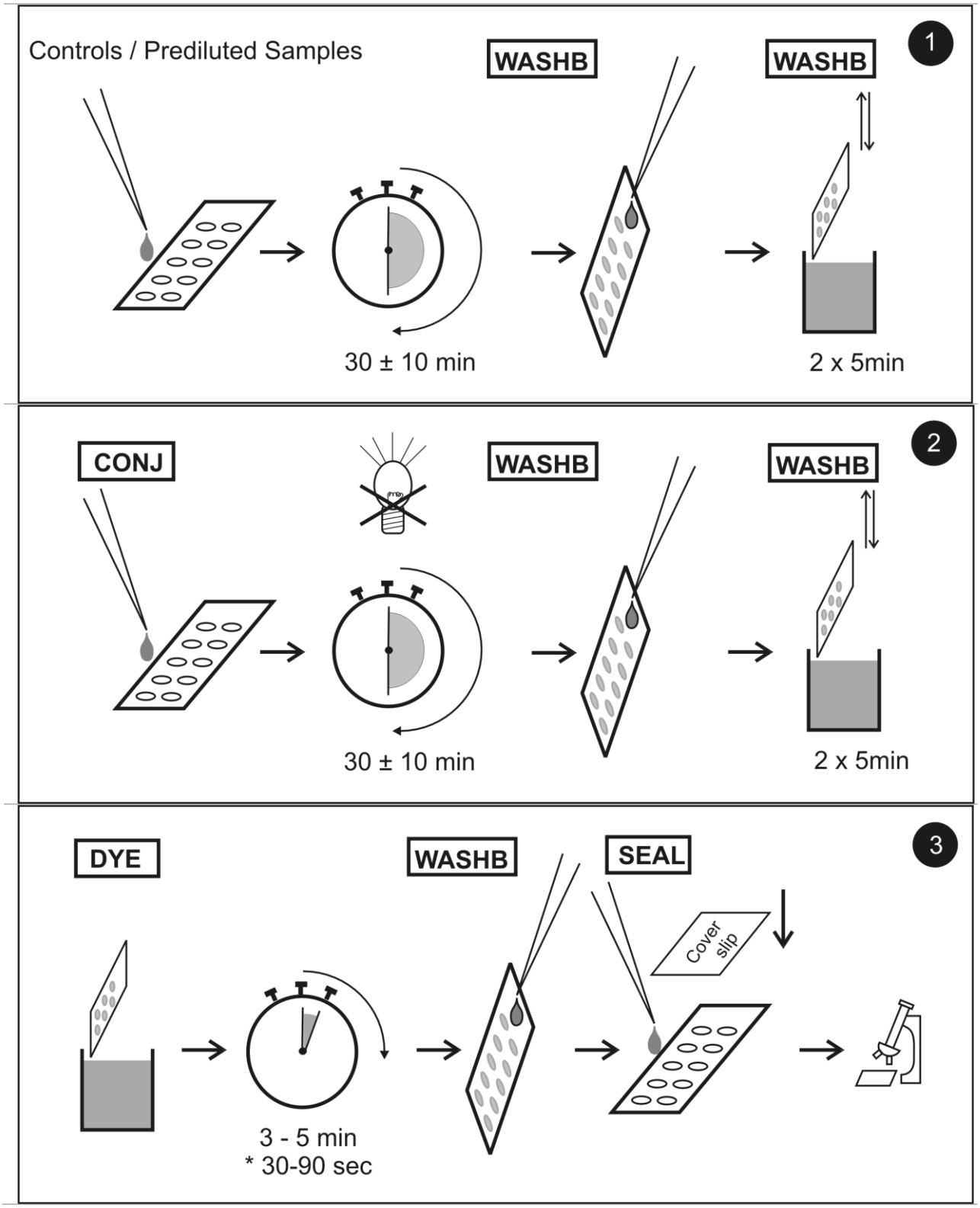
Allow all components to reach room temperature (20–26 °C / 68–78.8 °F) before use, mix well and follow the recommended incubation scheme for an optimum performance of the test.

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.

## f. 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.<br>Incubate slide(s) 30 minutes ± 10 minutes at room temperature in the moist incubator tray. Use consistent incubation times for the conjugate.<br>**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.<br>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.<br>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.<br>Lift slide(s) out of staining dish and carefully remove excess washing buffer.<br>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.<br>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.<br>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). |

## g. Work flow


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.<br>• 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.<br>• Microscope not adjusted.<br>• 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.<br>• Slide dried out.<br>• Lipemic, hemolytic sera.<br>• Microscope error. | • Check the washing instructions.<br>• Do not allow the slide to dry out.<br>• Use only fresh sera.<br>• 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 | |
| +2°C +8°C | - 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 | |
| Σ | - Numero di determinazioni | - Number of determnations |
| | - Nombre de déterminations | - Número de determinaciones |
| | - Anzahl der Prüfungen | - Αριθμός προσδιορισμών |
| | - Número de determinações | |