

IFA n-DNA - IgG

1. INTENDED USE

Immunofluorescence assay for the detection of IgG antibodies to native DNA in human serum and plasma with Crithidia luciliae

REF	VD0050	▽	50	IVD	kit
REF	VD0100	▽	100	IVD	kit
REF	VD0300	▽	300	IVD	kit
REF	VD0500	▽	500	IVD	kit

2. PRINCIPLE OF THE ASSAY

The detection of antibodies is based on the principle of an Indirect Immunofluorescence Assay (IFA). The slides are coated with Crithidia luciliae (inactivated). Any specific antibodies present in the patient's sample are bound during the first incubation. After removing unbound material by washing, the presence of specific antibodies is detected using Anti-Human -IgG conjugate during the second incubation.

Excess FITC conjugate is then removed. The formation of a stable three-part complex consisting of fluorescein antibody bound to human antibody, which is bound to antigen can be visualized with the aid of a fluorescence microscope.

3. DIAGNOSTIC RELEVANCE AND INTERPRETATION OF RESULTS

Antibodies to native DNA are highly specific for SLE.

Although low levels of nDNA antibodies may be detected by other autoimmune diseases e.g. Sjögren syndrome, Mixed Connective Tissue Disease (MCTD) and dermatomyositis, high titres are almost exclusively indicated by SLE.

Flagellates of the Crithidia luciliae species are particularly suited for the detection of specific antibodies to native DNA. These microorganisms possess the kinetoplast, a specific organ which stores DNA in high concentrations. Therefore a fluorescence of the kinetoplast is a highly specific indication for the presence of antibodies to native DNA.

A fluorescence of the nucleolus of Crithidia luciliae is caused by positive ANA reaction.

4. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

In tests to determine interlot and intralot reproducibility, the controls showed no discrepancies in staining patterns and staining intensity.

5. **Bib**

- R.J.T. Smeenk:
DNA as Antigen in SLE
Manual of Biological Markers of Disease
Kluwer Academic Publishers B 2.1 : 1-15 1994 Dordrecht
- R.L. Humbel :
Autoanticorps et Maladies Auto-Immunes
Elsevier 2^e edition 1997
- Wulf B. Storch:
Immunfluoreszenzfibel 2. Auflage
Blackwell Wissenschaftsverlag 1997

6. KIT COMPONENTS

Number and volume of the kit components are indicated on kit label.

1. SLIDES

SLIDES

slides coated with cell substrates

10x5 wells (VD0050); 10x10 wells (VD0100);
30x10 wells (VD0300); 50x10 wells (VD0500)

2. FITC ANTI-HUMAN CONJUGATE

CONJ|FITC|IgG

ready-to-use anti-human IgG conjugate
preservative: <0,1% sodium azide.

2,0 ml (VD0050); 3,0 ml (VD0100); 3 x 3,0 ml (VD0300);
5 x 3,0 ml (VD0500)

3. NEGATIVE CONTROL

CONTROL-

human serum, ready to use
preservative: <0,1% sodium azide.

0,5 ml (VD0050); 1,0 ml (VD0100); 3,0 ml (VD0300); 2 x 2,5 ml (VD0500)

4. POSITIVE CONTROL

CONTROL+

human serum, ready to use
preservative: <0,1% sodium azide.
The titre is reported on the label

0,5 ml (VD0050); 1,0 ml (VD0100); 3,0 ml (VD0300); 2 x 2,5 ml (VD0500)

5. EVANS BLUE

EVBL

ready to use

3,0 ml (VD0050,VD0100); 3 x 3,0 ml (VD0300) 5x 3,0 ml (VD0500)

6. MOUNTING MEDIUM

MM

ready to use

3,0 ml (VD0050, VD0100); 3 x 3,0 ml (VD0300); 5x 3,0 ml (VD0500)

7. PBS-BUFFER

BUF|PBS

powder

2 x (VD0050, VD0100); 6 x (VD0300); 10 x (VD0500)

The safety data sheet (MSDS) is available upon request.

7. STORAGE AND STABILITY

Store all reagents at \pm 2-8°C. Protect them from intense light and do not freeze. The expiration date of each component is indicated on the respective vial label. Do not use reagents beyond the expiration date.

The diluted **BUF|PBS** is stable up to 4 weeks when stored at \pm 2-8°C. Use only **SLIDES** with an intact vacuum packaging.

8. MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

- distilled water
- graduated cylinder
- coplin jars or staining dishes
- plastic squeeze bottle
- test tubes for sample dilution
- cover slips (24x60 mm)
- volumetric pipette
- moist chamber
- timer
- fluorescence microscope with FITC system,
(excitation wavelength 490 nm, emission wavelength 510 nm)

9. WARNINGS OR PRECAUTIONS

SAFETY PRECAUTIONS

The IFA test is for [IVD] use only.

- Do not mix lot specific reagents, such as [SLIDES], controls and [CONJFITC] from different kit lots. [BUF|PBS], [MM] and [EVBL] can be used for all IFA tests.
- Seal all bottles properly after use in order to avoid bacterial contamination. All samples and kit components should be considered potentially infectious. All control samples have been tested for Hepatitis Bs antigen, anti-HIV I and II, anti-HCV (CE/FDA) and found to be negative.
- The coated [SLIDES] are inactivated. However, normal laboratory precautions should be maintained when handling with infectious material. Do not pipette by mouth.
- Avoid contact with skin and mucous membranes when handling reagents, which contain preservatives (see kit contents). Wash thoroughly with water in case of contact and possibly look up a doctor.
- Controls containing sodium azide may react with lead and copper plumbing, building up explosive metal acids. Flush with sufficient water when disposing of reagents.
- For disposal the legal regulations have to be followed.

10. SPECIMEN COLLECTION AND STORAGE

- Only qualified and well-trained employees should carry out the assay procedure.
- The instruction for use describes the applicable test method. In case of modification or applications others than the intended use, or the use of automatic processors, the user has to validate the procedure and take the responsibility for it.
- Microbial contaminated specimen may cause interference.
- Lipaemic, haemolytic or icteric samples should only be tested with reservations although in our testing no negative influence has been found.
- Suitable specimens are serum or plasma (heparinized, EDTA) samples obtained by standard laboratory techniques.
- The samples should not be heat-inactivated since non-specific results may occur.
- Patient samples should be stored at $\pm 2-8^{\circ}\text{C}$.
For long-term storage $\pm -20^{\circ}\text{C}$ or lower is recommended.
Avoid repeated freeze-thaw cycles.
- Note:** Diluted patient samples must be used on the same day.

11. REAGENT PREPARATION

Bring all reagents to room temperature prior to use!

Prepare [BUF|PBS]: Completely dissolve one vial of [BUF|PBS] in 1 litre of distilled water. Reconstituted buffer solution should have a pH of 7,3 to 7,6.

Dilution of samples: Dilute patient sample with [BUF|PBS] according to test demands (screening titre).

Controls are ready to use.

12. PIPETTING AND INCUBATION STEPS

Screening Titre **1:10**

Note! Do not allow wells to dry at any time during the test procedure.

- Take required [SLIDES] out of the foil packets shortly before use and identify slides using a permanent marking pen. Do not touch the wells.

First incubation:

Pipette 1 drop of each control and 20 – 50 μl of each diluted sample onto the respective wells (covered completely) being careful not to touch cell substrate with pipette tip. The controls should be carried out for every testrun. Place [SLIDES] into a well-closed moist chamber to prevent drying.

Incubate [SLIDES] 30 minutes at room temperature.

3. **First wash step:**

Rinse the [SLIDES] gently with [BUF|PBS], using a squeeze wash bottle. Do not focus the buffer stream directly onto the wells. To prevent cross contaminations avoid rinsing from one well across other wells. For ten-well slides run PBS-stream from the midline of the slides successive along both rows to the edge of the [SLIDES]. Soak the [SLIDES] three times for 5 minutes with [BUF|PBS] in a coplin jar or staining dish with [BUF|PBS].

4. **Second incubation:**

After the washing procedure, shake of excess [BUF|PBS] (if necessary use blotting paper to draw off excess fluid but do not touch the wells directly) and place [SLIDES] back into moist chamber. Immediately add 1 drop of [CONJFITC] to each well.

Incubate [SLIDES] 30 minutes at room temperature in the dark.

5. **Second wash step:**

see **First wash step**

6. **Counterstain:**

Add 5 drops [EVBL] to 100 ml [BUF|PBS] solution in a staining dish and stain [SLIDES] for 5 minutes. [EVBL] covers unspecific background fluorescence.

- Remove [SLIDES] from the staining dish, rinse briefly with [BUF|PBS], shake off the excess [BUF|PBS] and apply 2-3 drops of [MM] across the slides. Gently lower the coverslip from the bottom to the top of the slides, avoid air bubbles.

- Read [SLIDES] within 30 minutes at **10 x 100** total magnification, using a fluorescence microscope (FITC filtercombination). Avoid longer exposition of one field of vision to minimize bleaching of FITC fluorescence.

13. SUGGESTIONS FOR TROUBLESHOOTING

In case that the IFA instructions are followed strictly, the reagents are handled with care and the samples and reagents are pipetted carefully, the following kinds of errors can be avoided to a large extend.

ERROR	POSSIBLE CAUSES
Cross contamination	-Too much test material -Fluid remaining between the wells, should be carefully dried around outside edges if necessary
Too few cells or organisms	-Cell lysis following prolonged contact with deionised water (observe the wash procedure) -Buffer squirted directly on the substrate in the well (observe the wash procedure) -Proteolytic enzymes have attacked the substrate (inactivate sample) -In covering the monolayer was disturbed
Inhomogenous fluorescence	-sample dried in the well, fluorescence stronger at the edge (moist environment) -sample does not cover the test well (air bubbles/sample at the hole well) -Buffer crystals on the [SLIDES] (wash) -Microscope incorrectly adjusted (check the adjustment of the microscope) -Unsuitable immersion oil
Unclear picture	-too much [MM] or air pockets -Microscope is dirty
Little or no fluorescence	-Bacterial contamination of the sample or [CONJFITC] (check storage) -Microscope not adjusted -pH-value (7,3 – 7,6) of [BUF PBS] too low -[CONJFITC] exposed to light (store [CONJFITC] protected from the light)
Background fluorescence	-[SLIDES] dried in a hot air stream (do not use a hairdryer and do not let the wells dry) -Lipaemic, haemolytic or bacterial contamination sample (use only fresh sample) -Marking the [SLIDES] with a wax pencil produces a film on the [SLIDES] (use a waterproof glass marker)

14. VALIDITY OF THE ASSAYS

All controls should be carried out with every test run. The fluorescence intensity of the **CONTROL+** is shown in the Quality Control Certificate. If controls give invalid levels then results from test samples are invalid too and retesting is required.

15. READING OF THE RESULTS

Fluorescence intensity

Read **SLIDES** at 10 x 100 total magnification, using a fluorescence microscope (FITC filtercombination).

The fluorescence intensity may be semi-quantified following our introduction:

3+ bis 4+	= maximal fluorescence, brilliant yellow-green
2+	= less brilliant yellow-green fluorescence
1+	= definite but dull yellow-green fluorescence
Equivocal – (+)	= very dim yellow-green fluorescence

The degree of intensity is not of clinically relevance and has only limited value as an indicator of titre. Differences in microscope optics, filters and light source may result in differences of +1 or more in intensity.

Fluorescence pattern of *Crithidia luciliae*

Kinetoplast (nDNA)	Nucleulus (ANA)	Result
+	+	positive
+	-	positive
-	+	negative

- = negative, + = positive

Negative Result

No characteristic fluorescence of kinetoplast. Nuclear staining is caused bei ANA.

Positive Result

Homogenous fluorescence of kinetoplast, respectively fluorescence of the kinetoplast and the nucleulus.

A. SEMIQUANTITATIVE TITRE

For the semi-quantification of the test results the **CONTROL-** and the **CONTROL+** must be tested with every test run. The fluorescent intensity of the sample can then be compared visually, or with the aid of an automatic processor, with the fluorescent intensity of the **CONTROL+**.

B. QUANTITATIVE TITRE

For quantification of the test results, the samples must be diluted to the endpoint titre. The endpoint titre is determined as the last dilution in which a **equivocal – (+)** reaction is visible.





Sample titration:

If the fluorescent intensity of the sample is < the fluorescent intensity of the Positive control, please dilute the sample to the endpoint titre of the Positive control (the titre is reported on the label)

If the fluorescent intensity of the sample is ≥ the fluorescent intensity of the Positive control, please dilute the sample higher than the endpoint titre of the Positive control (the titre is reported on the label)

Regarding diagnostic relevance and interpretation of results see **page 1**

16. Symbole nach IVD/ symbols used with IVD devices/ Symbole / Símbolos/ Simboli/ Simbolos/ Symboly

SLIDES	Objektträger/ slides/ lames/ portaobjetos/ vetrini/ lâminas
BUF/PBS	PBS-Puffer/ PBS-buffer/ Tampon PBS/ Buffer PBS / tampone PBS/ Tampão PBS
CONJ/FITC	FITC-Konjugat/ FITC conjugate/ Conjugue FITC/ conjugado FITC / FITC Coniugato/ Conjugado FITC
CONTROL+	Positive Kontrolle/ positive control/ Contrôle positif/ control positivo/ controllo positivo/ controle positivo
CONTROL-	Negative Kontrolle/ negative control/ Contrôle négatif/ control negativo/ controllo negativo/ controle negativo
MM	Einschlussmedium/ Mounting medium/ Liquide de Montage/ Medio de Montaje/ Mezo di montaggio/ Meio de Montagem
Bib	Literatur/ Literature/ Littérature/ Bibliografia/ Bibliografia/ Literatura/
LOT	Charge/ lot/ Lot/ lote/ carcia/ lote
IVD	In-vitro-Diagnostikum/ in vitro diagnostic/ Diagnostic in vitro / diagnóstico In-vitro/
REF	Artikel Nr./ reference or order number/ Référence ou numéro de commande/ referencia o número de pedido/ codice di riferimento o di commissione/ referência ou número de encomenda
▽ 100	100 Bestimmungen/ tests/ testó / determinazioni/ testes
	Gebrauchsanweisung beachten/ consult instructions for use/ consulter le mode d'emploi/consultar las instrucciones de uso/ consultare le istruzioni per l'uso/ consultar instruçõesde uso
	Temperaturgrenzen/ temperature limitation/ Limites de température/ Limites de temperatura/ Limiti di temperatura/ Limites de temperatura/
	Verfallsdatum:/ expiry date/ date d'expiration/ Fecha de caducidad/ Data di decadenza/ Limite de validade/ Datum Expirace
	Hergestellt von/ manufactured from/ fabriqué par/ elaborado por/ fabbricato da/ produzido por



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Symbole nach IVD/ symbols used with IVD devices/ Symbole / Símbolos / Simboli / Símbolos IVD

[SLIDE]	Objektträger/ slides/ lames/ portaobjetos/ vetrini/ lâminas
[BUF PBS]	PBS-Puffer/ PBS-buffer/ Tampon PBS/ Buffer PBS/ tampone PBS/ Tampão PBS
[CONJ FITC]	FITC-Konjugat/ FITC conjugate/ Conjugue ITCF/conjugado FITC / FITC Conjugato/ Conjugado FITC
[CONTROL +]	Positive Kontrolle/ positive control/ Contrôle positif/ control positivo/ controllo positivo/ controle positivo
[CONTROL -]	Negative Kontrolle/ negative control/ Contrôle négatif/ control negativo/ controllo negativo/ controle negativo
[EVBL]	Evans Blue/ Evans Bleu/ Bleu Evans/ Azul de Evans/ Blue di Evan/ Azul de Evans
[MM]	Einschlussmedium/ Mounting medium/ Liquide de Montage/ Medio de Montaje/ Mezo di montaggio/ Meio de Montagem
[SPE DIL]	Probenverdünnungspuffer/ dilution buffer/ Tampon de dilution/ reactivo compensador/ soluzione tampone/ estabilizador de diluição
[LOT]	Charge/ lot/ Lot/ lote/ carcia/ lote
[IVD]	In-vitro-Diagnostikum/ in vitro diagnostic/ Diagnostico in vitro/ diagnóstico in-vitro/ In-vitro diagnostic/ diagnóstico In-vitro
[REF]	Artikel Nr./ reference or order number/ Référence ou numéro de commande/ referencia o número de pedido/ codice di riferimento o di commissione/ referência ou número de encomenda
[RTU]	gebrauchsfertig/ ready to use/ Prêt à l'emploi/ listo para su uso/ pronto per l'uso/ pronto para usar
S 100	100 Bestimmungen/ tests/ testis/ / determinazioni/ testes
I	Gebrauchsinformation beachten/ consult instructions for use/ consulter le mode d'emploi/ consultar las instrucciones de uso/ consultare le istruzioni per l'uso/ consultar instruções de uso
t	Temperaturgrenzen/ temperature limitation/ Limites de température/ Limites de temperatura/ Limiti di temperatura/ Limites de temperatura
e	Verfallsdatum/ expiry date/ date d'expiration/ Fecha de caducidad/ Data di decadenza/ Limite de validade
M	Hergestellt von/ manufactured from/ fabriqué par/ elaborado por/ fabbricato da/ produzido por

(D) IFA TESTDURCHFÜHRUNG

Patientenserum mit [BUF|PBS] entsprechend dem angegebenen Suchtitel verdünnen.

Kontrollen sind [RTU]!

Benötigte Anzahl [SLIDE] entnehmen und beschriften. [SLIDE] nur an den Rändern anfassen.
1. / 2. Inkubation in einer feuchten Kammer.

20µL (1 Tropfen) KONTROLLE UND VERDÜNNTE PATIENTENPROBE AUFTRAGEN

1. INKUBATION 30 min. t 23+/- 1°C

1. WASCHSCHRITT

Nach 1. Inkubation [SLIDE] 10min. in einer Küvette mit hergestelltem [BUF|PBS] waschen. Puffer nach 5min. wechseln. Auftragsstellen nicht austrocknen!

1Tropfen [CONJ|FITC] je Testfeld auftragen

2. INKUBATION 30MIN. t 23+/- 1°C

2. WASCHSCHRITT (siehe 1. Waschschrift)

Pro 150 ml hergestelltem [BUF|PBS] in der Küvette 5 Tropfen [EVBL] zugeben, [SLIDE] hineinstellen.

GEGENFÄRBUNG MAX. 5 MIN.

[SLIDE] kurz mit hergestelltem [BUF|PBS] abspülen. Zwischen die Testfelder kleine Tropfen [MM] geben. Deckglas auflegen (Luftblasen vermeiden).

Mit Fluoreszenzmikroskop (FITC-Filterkombination) bei 400-800-facher Vergrößerung beurteilen.

(GB) IFA ASSAY PROCEDURE

Dilute patient sera with [BUF|PBS] solution to the appropriate dilution for the screening titre.

Controls are [RTU]!

Remove required [SLIDE] from pouche, mark them. Do not touch the wells.
1. / 2. Incubation in a incubator tray.

20µL (1 drop) OF EACH DILUTED SERUM AND CONTROLS INTO THE APPROPRIATE WELLS

1. INCUBATION 30MIN. t 23+/- 1°C

1.WASH STEP

After 1. Incubation wash [SLIDE] 10min. in [BUF|PBS]. Change buffer solution after 5min. Slide wells do not dry out!

Cover each well with 1 drop of [CONJ|FITC]

2. INCUBATION 30MIN. t 23+/- 1°C

2.WASH STEP (see 1. WASH STEP)

Per 150 ml [BUF|PBS] solution, add 3-4 drops [EVBL] and immerse [SLIDE].

COUNTERSTAIN MAX. 5 MIN.

Rinse [SLIDE] with [BUF|PBS] solution. Add 2-3 drops of [MM] along the midline of each slide. Place coverslip in position (avoiding air pockets).

Read [SLIDE] immediately at 400-800x total magnification with a fluorescent microscope.

F

TECHNIQUE D'IFI

Amener les sera des patients à la dilution de screening adéquate en les diluant avec le [BUF|PBS].

Les contrôles sont [RTU] !

Enlever la [SLIDE] du sachet. Ne pas toucher les puits.

1. / 2. Les incubations se font en chambre humide.

Déposer 20µl (1 goutte) de chaque serum dilué ou des contrôles dans un puits

1. Incuber 30 minutes, à $\pm 23 \pm/- 1^{\circ}\text{C}$

Premier LAVAGE

Après incubation laver la [SLIDE] 10 min. dans

[BUF|PBS]

Changer le [BUF|PBS] après 5 min.

Ne jamais sécher les puits.

Déposer dans chaque puits 1 goutte de [CONJ|FITC]

2. Incuber 30 minutes à $\pm 23 \pm/- 1^{\circ}\text{C}$

Second LAVAGE (voir 1. Lavage)

Ajouter 5 gouttes d'[EVBL] par 150 ml [BUF|PBS] dans le dernier bain. Immerger les [SLIDE]

Contre-colorer max. 5 min.

Rincer les [SLIDE] avec du tampon [BUF|PBS]
Déposer 2-3 gouttes de [MM] sur la ligne médiane des lames. Déposer la lamelle couvre-objet en évitant les bulles

Lire les [SLIDES] immédiatement à 400-800x, au microscope à fluorescence

E

DESARROLLO TEST IFA

Diluir el suero del paciente con [BUF|PBS] en proporción adecuada para titulación de screening. Controles están [RTU] (listos para uso) !

Retirar el número adecuado de [SLIDE] de su envuelta, marcarlos. No tocar los pocillos.

Incubaciones 1 y 2 en placa de incubación

20µL (1 gota) de cada suero diluido y controles en los pocillos correspondientes

INCUBACIÓN 1, 30 min, $\pm 23^{\circ}\text{C} \pm/- 1^{\circ}\text{C}$

Primer LAVADO

Después del lavado. Incubar el [SLIDE] 10 min. con [BUF|PBS]. Cambiar el [BUF|PBS] cada 5 min. No dejar que los pocillos se sequen.

Cubrir cada pocillo con 1 gota de [CONJ|FITC]

INCUBACIÓN 2, 30 min. $\pm 23^{\circ}\text{C} \pm/- 1^{\circ}\text{C}$

Segundo LAVADO (Ver lavado 1)

Para 150 ml [BUF|PBS] añadir 5 gotas [EVBL] y sumergir el [SLIDE].

SEGUNDA TINCIÓN MAX. 5 MIN

Aclarar el [SLIDE] con solución [BUF|PBS]. Anadir 2-3 gotas de [MM] en el centro del porta. Cubrir con un cubre (evitando las burbujas)

Leer inmediatamente los [SLIDE] a 400-800x con un microscopio de fluorescencia.

I

TEST IFA - PROCEDURA

Diluire i sieri dei pazienti con soluzione [BUF|PBS] fino alla diluizione appropriata per il titolo screening. I controlli sono [RTU]!

Estrarre le [SLIDE] necessari dall'involucro,

sigillarli. Non toccare i pozzetti. Eseguire la 1ª e 2ª

incubazione in una camera da incubazione

20µL (1 goccia) di ogni siero diluito e dei controlli nei pozzetti

1ª incubazione, 30 minuti a $\pm 23 \pm 1^{\circ}\text{C}$

Prima fase di lavaggio

Dopo la prima incubazione lavare le [SLIDE] per 10 minuti in [BUF|PBS]. Cambiare il [BUF|PBS] dopo 5 minuti. Non lasciare che i pozzetti si seccino!

Coprire ogni pozzetto con una goccia di [CONJ|FITC]

2ª incubazione, 30 minuti a $\pm 23 \pm/- 1^{\circ}\text{C}$

Seconda fase di lavaggio (vedere la prima fase)

Ogni 150 ml di [BUF|PBS] aggiungere 5 gocce di [EVBL] e immergere i [SLIDE].

COLORAZIONE MAX 5 minuti

Risciacquare le [SLIDE] con [BUF|PBS].
Aggiungere 2-3 gocce di [MM] lungo la linea mediana di ogni vetrino. Mettere un coprivetrino in posizione (evitare la formazione di bolle d'aria).

Leggere immediatamente le [SLIDE] con un microscopio a fluorescenza usando un ingrandimento 400-800x.

P

TÉCNICA IFA

Diluir as amostras com solução [BUF|PBS] na diluição apropriada para a pesquisa do título.

Controles estão [RTU]!

Retirar os necessários [SLIDE] da embalagem, marcar.

Não tocar nos poços.

1./2. Incubar num suporte de incubação.

20µL (1 gota) DE CADA AMOSTRA DILUÍDA E DOS CONTROLOS NOS POCOS APROPRIADOS

1. INCUBAÇÃO 30MIN. a $\pm 23 \pm/- 1^{\circ}\text{C}$

1. PASSO DE LAVAGEM

Após 1. incubação lavar [SLIDE] 10min. em [BUF|PBS]
Mudar a solução tempo após 5 min.
Os poços das [SLIDE] não podem secar.

Cobrir cada poço com 1 gota [CONJ|FITC]

2. INCUBAÇÃO 30MIN. $\pm 23 \pm/- 1^{\circ}\text{C}$

2. PASSO DE LAVAGEM (Ver 1. passo de lavagem)

Por 150 ml solução [BUF|PBS], adicionar 5 gotas [EVBL] a imergir [SLIDE].

CONTRASTE MAX. 5 MIN.

Enxaguar [SLIDE] com solução [BUF|PBS].
Adicionar 2-3 gotas de [MM] ao longo da linha média de cada [SLIDE]. Colocar as lâminas em posição (evitar bolhas de ar)

Ler [SLIDE] imediatamente a 400-800x da ampliação total com um microscópio de fluorescência.