



## **FLUORESCENT nDNA TEST SYSTEM**

**For *in vitro* Diagnostic Use**  
**For Professional Use**

*INTENDED USE: This is an indirect fluorescent antibody test for the semi-quantitative detection of anti-nDNA antibody in human serum. This test system is to be used as an aid in the diagnosis of systemic lupus erythematosus.*

### **SUMMARY AND EXPLANATION OF THE TEST**

Antinuclear antibody (ANA) is a general term used to describe autoantibodies against various cell nuclear proteins. Early studies of these autoantibodies, using immunofluorescent techniques, revealed a select few nuclear protein specificities (1). Because of the high correlation of positive ANA with systemic lupus erythematosus (SLE), a negative ANA essentially ruled out the disease (2).

Although antibodies specific to DNA continue to show a high disease correlation with SLE (3), in recent years a number of nuclear (4) and cytoplasmic (5-7) macromolecules have been detected and associated with other connective tissue diseases (8-10). Because a number of these antibodies appear to be of diagnostic and/or prognostic use in progressive systemic sclerosis (11-12), mixed connective tissue disease (13-15), Sjögren's syndrome (16-17), polymyositis (18), and rheumatoid arthritis (19), ANA testing is now recognized as a general screening tool for connective tissue disease (20).

SLE patients may produce antibodies to a variety of nuclear antigens, but antibodies directed against Sm (Smith antigen) and nDNA show the highest correlation with disease (20). Antibodies directed against Sm demonstrate a speckled ANA staining pattern while antibodies directed against nDNA generally demonstrate a homogeneous ANA staining pattern. Although low levels of nDNA antibodies may be present in the serum of patients with rheumatoid arthritis, Sjögren's syndrome, progressive systemic sclerosis, dermatomyositis, discoid lupus erythematosus, and mixed connective tissue disease (21), high levels of nDNA antibodies are seen almost exclusively in SLE. Antibodies against nDNA are thought to be involved in the pathogenesis of the most severe variants of SLE when deposited as immune complexes (22). Antibodies to nDNA occur in high titer, and, because they correlate with disease activity (23), their detection is important in the management of SLE patients.

Several assays are available for the detection of nDNA antibodies. The most commonly used methods include indirect immunofluorescence, radioimmunoassay, counterimmunoelectrophoresis, and immunodiffusion (24-27). The Immuno Concepts nDNA test system is an indirect fluorescent antibody (IFA) method. Serum antibody, reactive to nDNA, is detected by staining of the kinetoplast within the organism *Crithidia lucilliae* (34). *C. lucilliae* is a parasite of the blowfly and is non-pathogenic to humans. The kinetoplast of these hemoflagellates is part of the large mitochondrion in which the helical nDNA is concentrated (33-34). In electron micrographs, the kinetoplast appears as a slightly concave, disc-shaped structure containing mitochondrial cristae and a fibrous DNA mass (35). The kinetoplast is found between the centrally located nucleus and the basal body of the flagellum. Because the kinetoplast nDNA contains no single-stranded DNA (ssDNA) contaminants, potential problems of ssDNA false-positive reactions, which may occur with calf thymus DNA radioimmunoassay, are virtually eliminated (28-33).

# PRINCIPLE OF THE TEST

The Immuno Concepts nDNA test uses the indirect fluorescent antibody technique first described by Weller and Coons (36). Patient samples are incubated with antigen substrate to allow specific binding of autoantibodies to kinetoplast nDNA. If nDNA antibodies are present, a stable antigen-antibody complex is formed. After washing to remove non-specific antibodies, the substrate is incubated with an anti-human antibody reagent conjugated to fluorescein. When results are positive, there is the formation of a stable three-part complex consisting of fluorescent antibody bound to human anti-nDNA antibody, which is bound to nDNA antigen. This complex can be visualized with the aid of a fluorescent microscope. In positive samples, the kinetoplast or the kinetoplast and nucleus will show a bright apple green fluorescence within the *Crithidia luciliae* organisms. If the sample is negative for nDNA, the kinetoplast will show no fluorescence.

## SYSTEM COMPONENTS - MATERIALS PROVIDED

**Use:** All components come ready to use with no aliquoting or reconstitution required (except the PBS buffer which must be dissolved in deionized or distilled water before use).

**Storage:** All components can be stored under refrigeration at 2-10°C. After reconstitution, PBS buffer should be stored in screw cap containers and store between 2-25°C.

**Stability:** All components remain stable at least 12 months from date of manufacture. Do not use any component beyond its expiration date.

### REACTIVE REAGENTS

**Substrate Slides** **SLIDE**: nDNA substrate slides using *Crithidia luciliae* stabilized directly on the test wells. Unique moat slide design minimizes cross contamination of wells during testing. The slide pouch is filled with an inert non-toxic gas that contributes to the stability of the cells.

**Positive Control** **CONTROL|+**: Catalog No. 3021. Ready-to-use dropper vial containing 1.0 ml positive human control serum with antibody specific to nDNA antigens. This serum demonstrates a bright positive staining reaction on the kinetoplast on Immuno Concepts' *Crithidia luciliae* substrate.

**Titrateable Control Serum** **TC**: Catalog No. 3026. Ready-to-use vial containing 0.5 ml positive human control serum to be treated as an undiluted patient sample. See vial label for titer value.

**Negative Control Serum** **CONTROL|-**: Catalog No. 3031. Ready-to-use dropper vial containing 1.0 ml negative human control serum. The negative control serum will not show any specific staining of the kinetoplast on Immuno Concepts' *Crithidia luciliae* substrate.

**Fluorescent Antibody Reagent** **CONJ|FITC**: Catalog No. 3009 (9.0 ml), 3075 (23 ml). Anti-human IgG (heavy and light chains) conjugated to fluorescein isothiocyanate (FITC). Reagent comes ready-to-use in precision dropper bottles with 9.0 ml for each 10 slides in complete test kits.

### NON-REACTIVE COMPONENTS

**PBS Buffer Powder** **PWDR|PBS**: Catalog No. 1011. Phosphate-buffered saline powder (0.01 M, pH 7.4 ± 0.2). Each pouch contains sufficient buffer powder to make 1 liter. (One pouch of buffer powder is supplied for each five slides in complete test kits.)

**Preparation:** Dissolve one pouch of buffer powder in 1 liter of deionized or distilled water, cover, and store between 2-25°C for up to four weeks or until signs of contamination or other visible changes occur.

**Semi-Permanent Mounting Medium** **SOLN|MM**: Catalog No. 1111. Ready-to-use dropper vial containing 5.0 ml glycerol-based mounting medium.

**Coverslips** **CVSLP**: Catalog No. 1042. Each packet contains ten 24 x 64 mm No. 1 glass coverslips.

## ADDITIONAL MATERIALS REQUIRED - BUT NOT PROVIDED

Volumetric pipettes to deliver 20-25 µl volumes  
Coplin jars or staining dishes  
Squeeze bottle or Pasteur pipettes  
Serological pipettes  
Deionized or distilled water  
Test tubes to prepare serum dilutions  
Bibulous paper or paper towels  
Disposable gloves  
One-liter screw cap containers (for PBS buffer)  
Lab timer  
Fluorescent microscope equipped with 495 nm exciter filter and 515 nm barrier filter

## PRECAUTIONS

1. All human source material used in the preparation of controls for this product has been tested and found to be negative (not repeatedly reactive) for antibody to human immunodeficiency virus-1 and human immunodeficiency virus-2 (HIV-1 & HIV-2), antibody to hepatitis C virus (HCV), and for hepatitis B surface antigen (HBsAg) by an FDA approved method. No test method can offer complete assurance that HIV-1, HIV-2, hepatitis C virus, hepatitis B virus, or other infectious agents are absent. Thus, all control sera should be handled in the same manner as potentially infectious materials.
2. All patient samples should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health Manual: *Biosafety in Microbiological and Biomedical Laboratories, 1999 Edition*.
3. Dilution of the components or substitution of components other than those provided in this system may yield inconsistent results.
4. Sodium azide (0.09%) is used as a preservative. Sodium azide may react with lead or copper plumbing and form explosive metal azide salts. When disposing of reagents, flush with ample volumes of tap water to prevent potential residues in plumbing. Sodium azide is a poison and may be toxic if ingested.
5. This kit is for *in vitro* diagnostic use.
6. In the event hemolyzed or lipemic sera must be used, heat inactivate sera 30 minutes at 56°C for optimal results. Microbially contaminated sera should not be used.
7. The titratable control serum is intended for use in monitoring lot-to-lot and run-to-run reproducibility. It is not intended as a measurement of overall sensitivity or specificity of the assay.
8. Do not smoke, eat, or drink in areas where specimens or kit reagents are handled.
9. Avoid splashing or generation of aerosols at all times.
10. Incubation times and temperatures other than those specified may give false results.
11. Cross contamination of reagents or samples may give false results.
12. Reusable glassware must be washed and thoroughly rinsed free of detergents prior to use. All glassware must be clean and dry before use.
13. Bring all reagents, slides, and specimens to room temperature (18-25°C) prior to use.
14. Wear disposable gloves when handling specimens and reagents, and wash hands thoroughly afterwards.
15. Microbial contamination of reagents or samples may give false results.
16. Never pipette by mouth and avoid contact of reagents and specimens with skin and mucous membranes. If contact occurs, wash with a germicidal soap and copious amounts of water.

## SPECIMEN COLLECTION

**Collection:** Serum is the preferred specimen. Approximately 5 ml of whole blood should be collected aseptically by venipuncture using a sterile vacuum collection tube or other suitable collection system. Allow blood to clot at room temperature (18-25°C). Serum should be separated from the clot by centrifugation as soon as possible to minimize hemolysis.

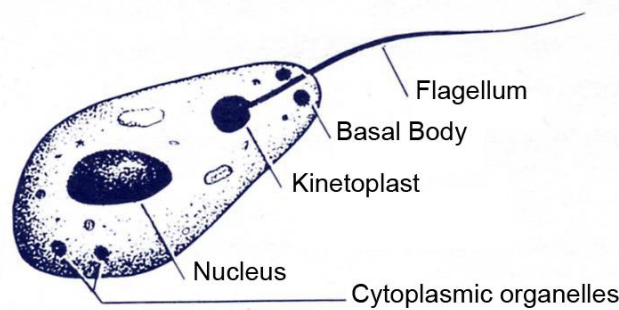
**Interfering Substances:** Sera exhibiting a high degree of hemolysis, icterus, lipemia, or microbial growth should not be used because these conditions may cause false results. Specimens containing visible particulate matter should be clarified by centrifugation before testing.

**Storage:** Sera may be stored at 2-10°C up to one week. If testing is further delayed, sera should be stored frozen at 20°C or lower. Serum should not be stored in a self-defrosting refrigerator or freezer.

**CAUTION:** Repeated freeze/thawing of patient samples may yield false positive or false negative results.

## INTERPRETATION OF RESULTS

Proper interpretation of results depends on clear recognition of the various morphologic features of the *Crithidia luciliae* organism.



The outer covering on most protozoa consists of a pellicle layer composed of lipoprotein. Inside the pellicle lies the plasma membrane. The plasma membrane encloses the cytoplasm consisting of a) an outer ectoplasm layer containing the basal body and flagellum and b) the endoplasm, a very fluid inner cytoplasm containing the nucleus, kinetoplast, and other organelles.

The pellicle, plasma membrane, basal body, and flagellum are generally considered permanent fixtures within the organism with little variability in location from cell to cell. Although the kinetoplast is generally located closer to the basal body than the nucleus, the exact location of this organelle may vary from cell to cell due to the fluid nature of the endoplasm.

In order to clearly differentiate the kinetoplast from the nucleus, view the positive control well. The kinetoplast will always be located nearer the flagellum (illustrated above). The negative control well will show no kinetoplast staining while the positive control will show kinetoplast staining.

READ ONLY SINGLE, WELL-DEFINED ORGANISMS WITHIN EACH FIELD. MORPHOLOGY MAY VARY FROM ORGANISM TO ORGANISM DUE TO FIXATION DURING LOG PHASE GROWTH.

### QUALITY CONTROL

Positive, negative, and PBS controls should be tested one time per run. The positive control should show bright apple-green fluorescence in the kinetoplast of the *Crithidia luciliae*, with or without staining of the nucleus. The negative control will show no staining of the kinetoplast. The PBS control is used to observe non-specific staining by the antibody reagent, and should not exhibit any green fluorescence. If the controls do not appear as described, the test is invalid and should be repeated.

### OPTIONAL TITRATABLE CONTROL

When reading titers, many laboratories begin reading with the well that contains the most dilute sample and read "backwards" to the 1:10 dilution. The first well in which clearly discernible kinetoplast staining is visible is the titer end-point. We recommend this technique for determining titer end-points.

The mean titer and titer range ( $\pm$  one dilution on either side of the mean) determined for this lot number were established in our laboratory and are stated as a guide. This control is provided to allow each laboratory to assess the reproducibility (precision) of its nDNA testing. Since this control is not intended to be an indicator of titer accuracy, each laboratory should establish its own mean titer end-point for this sample, and should use this information to assess run-to-run reproducibility (precision).

Through multiple testing of this titratable control, using the Immuno Concepts Fluorescent nDNA Test System, a mean titer value has been established for each lot number. The lot number, mean titer and titer range ( $\pm$  one twofold dilution on either side of the mean) are stated on the vial label and should be used as a guide for the test system performance.

The values obtained in our laboratory may differ from your values. Some of the many factors that can affect your results may include, but are not limited to:

1. The type of light source used. Mercury light sources will produce greater excitation energy at 495 nm than Quartz/Halogen. The 50-watt, 100-watt, and 200-watt mercury light sources differ little in excitation energy at 495 nm. The 100-watt Quartz/Halogen light sources will produce greater excitation energy at 495 nm than 50-watt Quartz/Halogen.

2. The condition and age of the light source. This is particularly true for mercury light sources, which generally exhibit a gradual reduction in excitation energy at 495 nm prior to burning out. This gradual reduction in excitation energy can result in a significant loss in sensitivity over several weeks. This problem can be avoided by keeping a time log. For best results, replace 50-watt mercury bulbs at 100 hours, and 100 or 200-watt mercury bulbs at 200 hours. Quartz/Halogen light sources generally do not exhibit a gradual reduction in excitation energy prior to burning out.
3. The type of exciter filter used. Interference exciter filters provide greater sensitivity over a much narrower wavelength than absorption exciter filters. Refer to your fluorescent microscope manual or sales representative for more information.
4. Proper alignment of the microscope light path. Refer to your fluorescent microscope manual for instructions.
5. The numerical aperture of the objective. With incident light fluorescence (Epi), fluorescence is increased exponentially as the numerical aperture (NA) of the objective is increased additively. This may cause a 40X objective with a NA of 0.65 to read one or more dilutions lower than a 40X objective with a NA of 0.85. The numerical aperture is printed on the side of the objective.
6. Suppression filters. Suppression filters reduce specific wavelengths of excitation and may be used in reducing sensitivity. Refer to your fluorescent microscope manual or sales representative for more information.
7. Precision and accuracy of dilution technique, equipment, and performance of the test procedures.

### INTERPRETATION OF PATIENT RESULTS

400X total magnification is recommended for viewing the *Crithidia*.

**Negative:** A serum is considered negative for antibodies to nDNA if kinetoplast fluorescence is less than or equal to the negative control well. Nuclear staining, without kinetoplast staining, is also considered negative for antibodies to nDNA.

**Positive:** A serum is considered positive if the kinetoplast shows clearly discernible staining with fluorescence greater than the negative control well.

**Titers:** When reading titers, many laboratories begin reading with the well that contains the most dilute sample and read "backwards" to the 1:10 dilution. The first well in which clearly discernible staining of the kinetoplast is visible is the titer end-point. We recommend this technique for determining titer end-points.

## FLUORESCENT INTENSITY

Fluorescent intensity may be semi-quantitated by following the guidelines for fluorescent antibody reagents established by the Centers for Disease Control and Prevention, Atlanta, Georgia (CDC).

- 4+ Brilliant yellow-green (maximal fluorescence)
- 3+ Less brilliant yellow-green fluorescence
- 2+ Definite cell pattern but dim fluorescence
- 1+ Very subdued fluorescence

A standard slide for the determination of these fluorescent intensities, FITC QC Slide™, catalog number 1900, is available from Immuno Concepts N.A., Ltd.

## REPORTING OF RESULTS

**Screening:** Results should be reported as positive or negative at the 1:10 dilution.

**Titering:** Results should be reported as the last serial dilution in which clearly discernible staining of the kinetoplast is seen. Results with a strong reaction at the 1:640 dilution should be reported as greater than 1:640.

### STAINING CHARACTERISTICS

**Kinetoplast:** A smooth or peripheral staining of the kinetoplast located near the flagellar region of the organism.

*Result:* Positive for antibodies to nDNA.

*Antigens:* nDNA.

*Disease Association:* High titers suggestive of active SLE (20) or in the case of previously diagnosed SLE, recurrent disease, or lack of response to therapy (21-23).

**Nucleus:** A smooth, peripheral, or speckled staining of the nucleus.

*Result:* Negative for antibodies to nDNA.

*Antigens:* Nuclear associated antigens (21-23).

*Disease Association:* Non-specific connective tissue disease may be indicated by the positive nuclear staining.

**NOTE:** Positive ANA results by HEp-2 or other substrates do not normally give the corresponding nuclear staining on *C. luciliae*, e.g. a speckled ANA by HEp-2 does not demonstrate speckled nuclear staining on *C. luciliae*.

**Basal Bodies:** A smooth staining of two spheres located where the body of the organism attaches to the flagellum in the ectoplasm.

*Synonyms:* Basal feet.

*Results:* Negative for antibodies to nDNA.

*Antigens:* Basal body associated antigens.

*Disease Association:* Reported in SLE patients not exhibiting kinetoplast or nucleus staining (37).

**Flagellum:** Staining of the flagellum of the organism.

*Synonyms:* Tail region of the organism.

*Result:* Negative for antibodies to nDNA.

*Antigens:* Unknown flagella-associated antigens.

*Disease Association:* Unknown.

## LIMITATIONS OF THE TEST

1. Diagnosis cannot be made on the basis of anti nDNA antibody detection alone. The physician must interpret these results in conjunction with the patient's history and symptoms, the physical findings, and other diagnostic procedures.
2. Treatment should not be initiated on the sole basis of a positive test for anti nDNA antibodies. Clinical indications, other laboratory findings, and the physician's clinical impression must be considered before any treatment is initiated.
3. Certain drugs, including procainamide and hydralazine, may induce a lupus erythematosus-like disease. Patients with drug-induced LE may demonstrate positive ANAs commonly directed against nuclear histones, although antibody to nDNA has also been reported (38-39).
4. Although a high-titered nDNA may be highly suggestive of SLE, it should not be considered diagnostic but rather viewed as a part of the overall clinical history of a patient. Low titers of nDNA antibodies are often present in the sera of patients with rheumatoid arthritis, Sjögren's syndrome, progressive systemic sclerosis, dermatomyositis, discoid lupus erythematosus, and mixed connective tissue disease (21).
5. Because of the many options available in fluorescent microscopes, it is recommended that light sources, filters, and optics be standardized when comparing patient titers between laboratories.
6. Patients undergoing steroid therapy may have negative results for nDNA antibody (40).

## EXPECTED VALUES

The expected value in the normal population is negative at a 1:10 screening dilution. Certain drugs, such as hydralazine, may induce nDNA antibody production (38-39).

## PERFORMANCE CHARACTERISTICS

The Immuno Concepts nDNA test system was evaluated in comparison with two other fluorescent antibody tests in commercial distribution (41). The study employed 103 serum samples from normal individuals as well as from patients with diagnoses including systemic lupus erythematosus (SLE), mixed connective tissue disease (MCTD), Raynaud's-progressive systemic sclerosis-CREST variant (PSS-CREST), rheumatoid arthritis (RA), juvenile rheumatoid arthritis (JRA), as well as other connective tissue disease. Sera were tested at the recommended screening dilutions for each manufacturer. Study results are summarized in Table 1.

TABLE 1

DIAGNOSIS	Number of Patients	Immuno Concepts Positive 1:10	Manufacturer A Positive 1:10	Manufacturer B Positive 1:10
SLE	30	13	13	11
MCTD/overlap	6	0	0	0
Raynaud's PSS-CREST	17	0	0	0
RA	2	0	0	0
JRA	4	0	0	0
Other connective tissue disease	9	0	0	0
Hospitalized controls	11	1	1	1
Normal controls	24	0	0	0

The hospitalized control that was positive on all *Crithidia lucilliae* nDNA tests had immune complex renal disease, which did not meet criteria for diagnosis of SLE.

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In the event of damage to the protective packaging, please contact Immuno Concepts prior to use.



Manufacturer



Authorized Representative in the European Community



Temperature Limitation



Contains Sufficient for <n> tests



Consult Instructions for Use



In Vitro Diagnostic Medical Device



MDSS GmbH  
Schiffgraben 41  
D-30175 Hannover, Germany



Immuno Concepts, N.A. Ltd. 9825 Goethe Road, Suite 350 Sacramento, CA. 95827  
 Technical Support USA: 1.800.251.5115 Outside USA: 1.916.363.2649  
 Email: [technicalsupport@immunoconcepts.com](mailto:technicalsupport@immunoconcepts.com)

# FLUORESCENT nDNA TEST PROCEDURE

**NOTE: If the laboratory is using an automated sample processing system, the procedure and recommendations of the manufacturer of the processor should be followed. The slide processing system should be programmed for the appropriate sample dilutions, dispense volumes, and incubation times as outlined below.**

- 1. RECONSTITUTE BUFFER (PBS)**  
Dissolve contents of one buffer pouch in one liter of deionized or distilled water. The PBS buffer may be covered and stored at 2-25°C up to four weeks.
- 2. DILUTE PATIENT SAMPLES**  
Screening: Dilute patient samples to 1:10 by adding 0.1 ml (100 µl) serum to 0.9 ml reconstituted PBS.  
Semi-Quantitative Titering: To make two-fold serial dilutions of screening samples (e.g. 1:20, 1:40, 1:80...1:640), remove 0.5 ml of the 1:10 dilution and mix with 0.5 ml of PBS to achieve a 1:20 dilution, and continue serial dilutions in this fashion.
- 3. DILUTE OPTIONAL TITRATABLE CONTROL**  
Treat the optional titratable control as an undiluted patient sample. Dilute the control 1:10 by adding 0.1 ml (100 µl) of the control serum to 0.9 ml of reconstituted PBS. Make two-fold serial dilutions of the titratable control as outlined above.
- 4. PREPARE SUBSTRATE SLIDES (20-25 µl/well)**  
Remove slide(s) from pouch(es) and place control sera on control wells as follows: Invert control dropper bottle and squeeze gently until drop is visible at the tip. Gently touch the drop to appropriate control well while avoiding direct contact of dropper tip with slide surface. Add 1 drop (20-25 µl) patient sample to the numbered wells.  
**CAUTION: DIRECT CONTACT OF DROPPER TIP WITH SLIDE SURFACE MAY RESULT IN DAMAGE TO THE ANTIGEN SUBSTRATE.**
- 5. INCUBATE SLIDES (30 ± 5 minutes at room temperature, i.e. 18-25°C)**  
Place slide(s) into a moist covered chamber (a petri dish with moistened paper toweling will be adequate). Incubate, with lid in place, for 30 minutes (± 5 minutes) at room temperature (18-25°C).
- 6. PBS RINSE**  
Remove slide(s) from incubator tray and rinse briefly with PBS using a squirt bottle, Pasteur, or serological pipette. Do not squirt buffer directly on the wells.  
**NOTE:** To avoid cross contamination on 13-well slides, direct PBS stream along midline of slide, tilting first toward wells 1-5 followed by tilting toward wells 6-10.
- 7. PBS WASH (10 minutes)**  
Wash slide(s) 10 minutes with PBS in a slide staining dish or Coplin jar. This wash may be extended 10-30 minutes with no variability in final test results. Discard PBS wash solution after use.
- 8. FLUORESCENT ANTIBODY REAGENT (Cover the wells with 10-12 drops)**  
Remove one slide at a time from PBS and dip 3-5 times in deionized or distilled water. Tap slide on its side against bibulous paper or paper toweling to remove excess water. Immediately return slide to the incubation chamber and cover the wells completely using fluorescent antibody reagent; begin by placing a drop over each well. Repeat for each slide. Fluorescent antibody reagent has been titered to compensate for residual deionized or distilled water remaining on the slide after rinsing.  
**NOTE:** It is important that slide wells do not dry during this procedure or damage to the substrate may occur.  
**DO NOT BLOT OR DRY THE SLIDE IN ANY MANNER OR ALLOW SLIDE TO SIT WITHOUT FLUORESCENT ANTIBODY REAGENT FOR LONGER THAN 15 SECONDS.**
- 9. INCUBATE SLIDES (30 ± 5 minutes at room temperature, i.e. 18-25°C)**  
Place lid on incubation chamber and cover with a paper towel to prevent exposure to light if the chamber is not opaque. Allow slide(s) to incubate 30 minutes (± 5 minutes) at room temperature (18-25°C).
- 10. PBS RINSE**  
Remove slide(s) from incubator tray and rinse briefly with PBS. Do not squirt buffer directly on the wells.
- 11. PBS WASH (10 minutes)**  
Wash slide(s) 10 minutes with PBS in a slide staining dish or Coplin jar. This wash may be extended 10-30 minutes with no variability in final test results.
- 12. MOUNT COVERSIP**  
Remove one slide at a time from PBS and dip 3-5 times in deionized or distilled water (Optional). Tap slide on its side against bibulous paper or paper toweling to remove excess water.  
**DO NOT BLOT OR DRY THE SLIDE IN ANY MANNER OR ALLOW TO SIT WITHOUT COVERSIP FOR LONGER THAN 15 SECONDS.** Add 4-5 drops of semi-permanent mounting medium along midline of each slide. Carefully place coverslip in position, avoiding air pockets, by gently lowering coverslip from one end of the slide to the other.  
**NOTE:** Excess mounting medium on slide may result in high background fluorescence, due to light scattering, or lack of clear resolution of cells (blurred image). Excess mounting medium may be removed from slide by gently blotting coverslip with bibulous or lens paper while avoiding any direct movement of the coverslip.

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