INSTRUCTIONS FOR USE

Miscellaneous IFA Reagents

Evans Blue counterstain	R001
Phosphate Buffered Saline (PBS)	R002
Phosphate Buffered Saline (PBS) - 10 litres	R003
FITC Mounting Medium	R005
FITC Mounting Medium	R006
ANCA Mounting Medium	R009

INTENDED USE
The Bio-Diagnostics IFA reagents listed above are intended for use in indirect immunofluorescent antibody (IFA) tests to detect autoantibodies in human serum.

SUMMARY AND EXPLANATION
These reagents are used during indirect immunofluorescent antibody (IFA) tests.
PBS is used to dilute human sera to an appropriate screening dilution and to wash slides between incubation stages.
FITC/ANCA Mounting medium is used to mount coverslips onto stained slides prior to viewing under a microscope.
Using Evans Blue counterstain, negative reactions lead to brownish-red patterns. The counterstaining intensity will vary according to the degree of counterstaining with the staining solution.

PRINCIPLE OF THE TEST
Human sera diluted in PBS are incubated on tissue sections. The primary test reaction involves circulating antibodies present in the patient's serum, which attach to their homologous antigens.
A rinsing period is followed by a secondary reaction using a FITC anti-human globulin conjugate. The antigen surface is then thoroughly rinsed free of unbound conjugate and a coverslip is mounted onto the slide. It is then viewed under an appropriate fluorescent microscope to visually identify various morphological patterns of nuclear fluorescence. With a positive reaction, the nuclear pattern appears apple-green when viewed under a fluorescent microscope, whilst a negative reaction appears black or greenish-black.

MATERIALS PROVIDED

IFA/DFA PBS	Buffer Pack no: R002 or R003
MM	Mounting Medium no: R005 or R006 or R009
EB	Evans Blue counterstain: R001

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WARNINGS AND PRECAUTIONS
1. Do not use reagents beyond their expiration date.
2. For in vitro diagnostic use.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED
Substrate section slides as appropriate
Negative and positive controls

Test tubes and rack or microtitre system	Disposable pipettes	Coverslips
Staining Dish and Slide Forceps	Moisture Chamber	Distilled Water
Volumetric Flask (500 ml)	Fluorescence Microscope	Paper Towels – lint free

All reagents required are available from Bio-Diagnostics Ltd - see catalogue for details.

REAGENT PREPARATION & TEST INSTRUCTIONS
1. Rehydrate PBS Buffer Pack in sterile distilled water as follows: R002 - rehydrate with 1 litre, R003 - rehydrate with 10 litres.
2. PBS is used for dilution of sera and for rinsing slides between incubation stages.
3. If counterstaining is required, add 5-10 drops of Evans Blue per 75ml of wash buffer for the first rinse.
4. Following staining, place 4-5 drops of mounting medium on slide, apply a coverslip and examine the slide under a fluorescent microscope.

KEY FOR OTHER SYMBOLS
Used in this instruction leaflet and on accompanying product packaging:

Manufacturer	Contains sufficient for <n> tests	RFU	Ready for Use
Temperature limitation		IVD	In vitro diagnostic medical device

STORAGE AND STABILITY
Mounting Medium should be stored at a temperature of +2ºC to +8ºC.
Phosphate Buffered Saline is stable at room temperature storage. The reconstituted Buffer does not contain preservatives and should be stored at 2-8°C. Care should be taken to avoid contamination.
The stability of the reagents is as indicated by the expiry date on the packaging under the above storage conditions. This applies to unopened and opened reagents.

QUALITY CONTROL
Positive and negative serum controls must be included in each day's testing to confirm reproducibility, sensitivity and specificity of the test procedure.

TEST LIMITATIONS
No diagnosis should be based upon a single serologic test result since various host factors must be taken into consideration.

Issue date: 08/07/2019