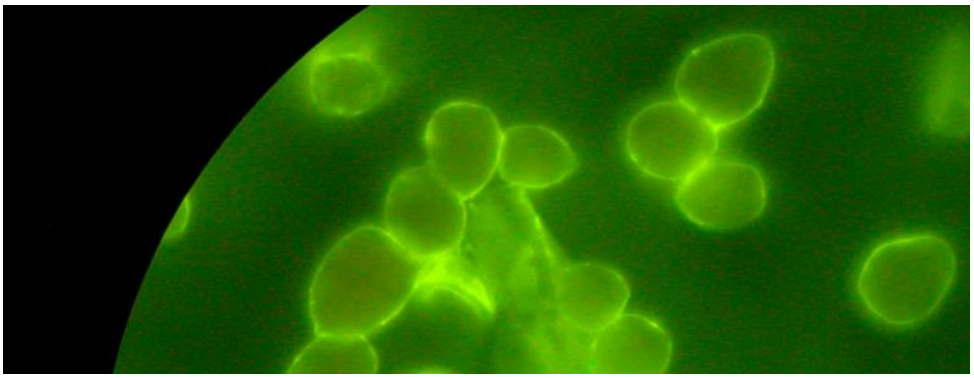
LOEWE®

Protocol for Immunofluorescence (IF) Assay



LOEWE® Biochemica GmbH, Mühlweg 2a, D-82054 Sauerlach
Contact Germany Phone +49-(0)-81046 1620 E-mail: Service@loewe-info.com Web: www.loewe-info.com

Assay Principle

Immunofluorescence is a technique based on an antigen-antibody reaction where the antibodies are tagged (labelled) with a fluorescent dye and the antigen-antibody complex is visualized using ultra-violet (fluorescent) microscope. Therefore it allows the visualization of the distribution of the target molecule through the sample. This protocol describes how to perform an indirect immunofluorescence assay for plant pathogenic bacteria.

[1)]European and Mediterranean Plant Protection Organization (EPPO) PM 7/97 (1). Indirect immunofluorescence test for plant pathogenic bacteria.

Sample preparation

According to international or laboratory standards.

Formulations of Buffers

IF-Buffer [10 mM phosphate buffered saline (PBS), pH 7.2] For dilution of antibodies, antiserum and positive controls and washing the slides.	$Na_2HPO_4 \cdot 12H_2O$ 1.07 g $NaH_2PO_4 \cdot 2H_2O$ 0.4 g NaCl 8.0 g KCl 0.2 g Distilled water 1.0 l Dissolve ingredients, check pH and sterilize by autoclaving at 121°C for 15 min.
IF-Buffer-Tween This buffer is used for washing the slides.	Add 0.1% Tween 20 to the IF-Buffer.
Phosphate buffered glycerol (Cover solution), pH 7.6 This buffer is used as a mountant fluid on the windows of IF slides to enhance fluorescence.	$Na_2HPO_4 \cdot 12H_2O$ 3.2 g $NaH_2PO_4 \cdot 2H_2O$ 0.15 g Glycerol 50 ml Distilled water 100 ml

Handling and Storage of Reagents

The following conditions are recommended for long-term storage:

- Antisera: prepare suitable aliquotes and freeze at min. -20°C.
- Secondary antibodies: Store lyophilizated antibodies refrigerated until opened. For extended storage after rehydration, aliquot and freeze at min. -70°C. Alternatively, add en equal volume of glycerol for a final concentration of 50% and store at -20°C as a liquid. Note: adding glycerol reduces the stated protein concentration and dilution range by one-half.
- Positive Controls: Store lyophilized controls refrigerated: Before use reconstitute as indicated in the corresponding product specification sheet. Prepare aliquots and store them frozen until use.

As a general rule, repeated thawing and freezing should be avoided!

Assay Procedure

Dilution of antiserum:	according to the recommended working dilution*
Dilution of secondary antibody:	according to the recommended working dilution*
Dilution of the positive control:	according to the product specific data*

(* recommended working dilutions are stated in the corresponding product specification sheet)

Sample application

Pipette 20 µl of sample per window of the multiwell microscope slide and fix the samples by heating at 60°C for 20 minutes using a thermo-stated heating plate.

Application of antiserum

Apply 20 µl of the diluted antiserum per window. Incubate the slides in a humid chamber for 30 minutes at room temperature.

1st Washing step

Rinse the slides carefully with IF-Buffer-Tween. Wash the slides two times for 7 minutes with IF-Buffer. Rinse with distilled water and carefully remove excess moisture (e.g. drying in an incubator at 37°C).

Application of secondary labeled antibody

Apply 20 µl of the diluted secondary antibody per window. Incubate the slides **in the dark** (to avoid bleaching) in a humid chamber for 30 minutes at room temperature.

2nd Washing step

Rinse the slides carefully with IF-Buffer-Tween. Wash the slides two times for 7 minutes with IF-Buffer. Rinse with distilled water and carefully remove excess moisture (e.g. drying in an incubator at 37°C).

Evaluation

Pipette 5-10 µl of the phosphate buffered glycerol per window or distribute a sufficient amount across the slide, apply a coverslip and avoid exposure of the slides to excess light. Examine the slides on an epifluorescence microscope with suitable filter combination. Magnification: 400 – 1000 (immersion oil is necessary).