

primediagnosics
Plant Research International

Indirect ImmunoFluorescent colony staining (IIF) for detection of plant bacteria

Buffers and chemicals:

PBS 0.01M: 8 gr NaCl
 2.7 gr Na₂HPO₄ x 12H₂O
 1 gr KH₂PO₄
 0.5 gr NaN₃
 pH 7.4 with NaOH
 Add demineralized water to 1000 ml total volume

Inbed buffer: 3.2 gr Na₂HPO₄ x 12H₂O
 0.15 gr NaH₂PO₄ x 2H₂O
 0.05 gr NaN₃
 pH 7.6 with NaOH
 Add demineralized water to 100 ml total volume
 Add 50 ml glycerol for fluorescence microscopy

Inbed buffer: Vectashield mounting fluid (H1000 Brunswick)

Washing buffer: 0.001 M PBS (10 x dilutes 0.01 M PBS)

Polyclonal specific IgG raised in rabbit

GAR-FITC Sigma F0382

Equipment and materials:

Fluorescence microscope

Heating plate of 50°C

Object glasses with round wells of 4 mm diameter (Nutacon no.10-342)

Procedure:

1. Prepare a dilution series of the sample in 0.01 M PBS. False-negative reactions can occur when there are too many target bacteria in the sample. Do not forget a positive and negative control!
2. Use a pure culture for positive control. Dilute a solution of pure culture of the target bacteria to 10⁷, 10⁶ and 10⁵ cells/ml PBS.
3. Clean the glasses extensively with alcohol.
4. Bring 5 ul of a sample in a well of an object glass. Bring increasing concentrations on the glass from the left to the right.
5. Dry the glasses at 50°C on the heating plate.
6. Fixate the samples by keeping the glasses very short in the flame of a bunzen-burner.
7. Dilute the antiserum in 0.01 M PBS.
8. Add 5 ul of the diluted antiserum to every well.
9. Incubate the glasses 30 minutes in a moist, dark place at 27°C (e.g. horizontal on wet filter paper in a closed box in a stove).

10. Wash the glasses 2 times 5 minutes with washing buffer. For this pour or spray the buffer on the glass carefully, leave for 5 minutes and pour the buffer from the glass.
11. Wash the glasses 1 minute with demi water and dry them on the heating plate of 50°C.

12. Dilute the GAR-FITC 200 times in 0.01 M PBS
13. Add 5 ul of the diluted GAR-FITC to every well.
14. Repeat steps 9, 10 and 11.

15. Bring ca. 50 ul inbed buffer or 1 drop vectashield on every glass and put an object glass over this to spread the solution. Avoid air bubbles between the glasses.
16. Examine the samples under the fluorescence microscope with blue light (490 nm). Look for green fluorescent cells with the same morphology as the positive control. Use a magnification of 100 of 50 times.

17. Scoring the results:
 - No fluorescent cells = 0
 - Weakly fluorescent cells = 1
 - Moderate fluorescent cells = 2
 - Clearly fluorescent cells = 3
 - Brightly fluorescent cells = 4

Remarks

1. It is recommended to determine the optimal dilution of the antiserum by testing a dilution series of the antiserum before testing the plant samples.
2. When glasses with bigger wells are used the amount of sample should be increased.
3. The intensity of the fluorescence is dependent on the pH of the inbed buffer (optimal between pH 7.5 and 8.5).
4. Bring the samples on the glasses within 1 hour to prevent enzymatic destruction of the cells.
5. Swift drying and a thin layer of cells on the glasses favor an equal distribution of the sample.