QuickBead™ Kit for Bacterial Fruit Blotch

Catalog Number AB 048 BS

Intended Use

The EnviroLogix QuickBead Kit for Bacterial Fruit Blotch (BFB) enables sensitive testing for *Acidovorax avenae* subsp. *citrulli* (*Aac*), the causal agent of BFB in cucurbits. Immunomagnetic separation (IMS) of the pathogen using the QuickBeads, combined with PCR (IMS-PCR), facilitates reproducible detection of *Aac* down to $10^3 – 10^2$ cfu/mL.

How the Test Works

This test is intended for use on seedlots that are suspected to be infected with bacterial fruit blotch. The magnetic beads provided in the kit are coated with a monoclonal antibody that is very specific to *Aac.* The antibody has been shown to detect all known *Aac* strains, and it does not react with other *Acidovorax* spp, Pseudomonads, or cucurbit pathogens including *Acidovorax facilis, Acidovorax avenae* subsp. *avenae, Acidovorax konjaci, Acidovorax cattleyeae, Comamonas testosteroni, Burkholderia cepacia, Pseudomonas acidovorans,* and *Pseudomonas lachrymans.* These immunomagnetic beads (IMBs), therefore, allow sensitive and specific capture of *Aac* from seed extracts, expediting clean downstream analysis of the bacteria by conventional or real time PCR techniques.

Use of IMBs for IMS-PCR-based detection of BFB has been characterized and documented by Ron Walcott, Associate Professor of Plant Pathology at the University of Georgia. The methods described here are based a) on his reports (Walcott, R.R., *et al.* (2006), *Seed Sci. & Technol.*, **34**, 101-116 and Walcott, R.R., *et al.* (2000), *Plant Disease*, **84**, 470-474) and b) independent reports from seed companies.

Sample Preparation

See Precautions & Notes for instructions on preparing PBS solution.

Preparation of the seed wash:

1. Soak a 5,000-seed sample in approximately 500 mL 1X PBS (pH 7.4) in a flask, resealable plastic bag, or other suitable container, for at least one hour in an orbital shaker at 150 rpm.

   Note: Seed size will affect the weight of the 5,000-seed sample. To calculate the amount of PBS to add, multiply the weight of the 5,000-seed sample in grams by 2, then use the value obtained as the number of mL of PBS to add. For instance: 5,000 seeds weighing 250 g will require 500 mL of PBS.

2. Filter and collect the seed wash through four layers of cheesecloth, using a sterile funnel and a sterile 1L collection flask.

3. Centrifuge the seed wash for 10 minutes at 2500 rpm.

4. Decant supernatant into another 1L flask and add pectinase to a final 5% concentration (5 mL of pectinase to 95 mL of seed wash). Incubate for one hour at room temperature if possible, shaking at 100 rpm. Note: pectinase performs better under acid pH conditions (most seed washes are acidic).

5. Filter and collect the pectinase-treated seed wash using a Whatman #1 filter paper and another sterile 1L flask. If this filtering step takes one hour or more, replacing the filter can speed up the process.

Highlights:

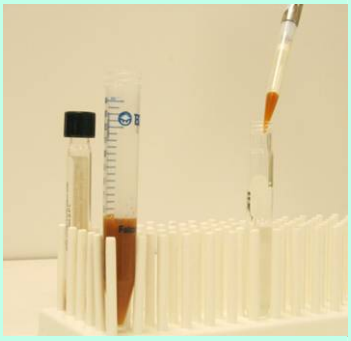
- *Rapid alternative to seedling grow-out*
- *Sensitive immunomagnetic capture*
- *Highly specific for* Acidovorax avenae *subsp.* citrulli

Contents of Kit:

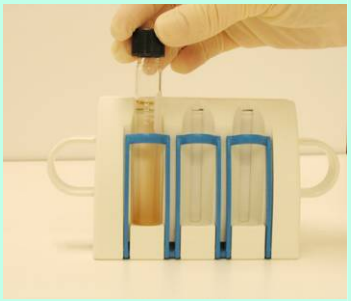
- *Antibody-coated beads in stabilizing buffer*
- *See "Precautions & Notes" for a list of materials and equipment needed for preparing samples*

Soak seeds in PBS, then filter

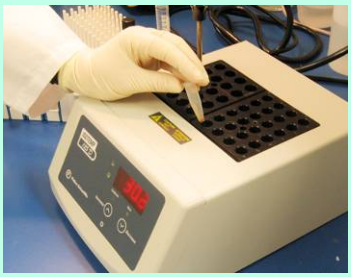
Resuspend settled QuickBeads



Add 0.25 mL QuickBeads to 6 mL of the extracted sample



Collect beads using magnetic particle concentrator



Heat QuickBeads on heat block to release DNA

Concentration of the seed wash:

6. Centrifuge the filtered seed wash for 15 minutes at 8,000 rpm. Pellet will form on the side of the centrifuge bottle.
7. Eliminate the supernatant, re-suspend pellet in 6 mL 1X PBS, and place it into a glass screw-cap test tube (e.g. No. 9825, Pyrex, USA). Use this sample for immunomagnetic separation as described in the next section.

Immunomagnetic Separation

See Precautions & Notes for instructions on preparing PBS-Tween and Tris-Tween solutions.

1. Repeatedly invert a tube of QuickBeads to completely resuspend the settled beads.
2. Pipette 0.25 mL of resuspended beads to the 6 mL of concentrated seed extract prepared in Step 7 above. Incubate sample for one hour with end-over-end mixing at 4°-8°C (mix/agitate at ~30 rpm). Use an end-over-end mixer or the orbital shaker used in the seed wash steps.
3. Collect beads using a magnetic particle concentrator (MPC; e.g. Dynal MPC-L from Invitrogen). Wash four times; three times with 8 mL 1X PBS-Tween, then once with 8 mL of 10mM Tris-Tween. Use the MPC to separate the beads from the washing solution. Avoid disturbing (agitating) the beads as much as possible.
4. For maximal concentration: After the last wash, add a small volume of 10mM Tris-Tween to the glass tube to precipitate all the beads to the bottom. Using a pipette, transfer all the beads to a 1.5 mL plastic microcentrifuge tube. Remove the excess of Tris-Tween from the tube using the MPC, then re-suspend beads in 20-50 µL of 10mM Tris-Tween.
5. To lyse bacteria and release DNA, heat the closed tube in 100°C heat block for 10 minutes. Next, centrifuge samples at 13,000 rpm for 15 seconds to collect the QuickBeads at the bottom. Place the tubes back in the MPC to facilitate removal of DNA from the supernatant without collecting beads. Use the DNA released for PCR. Place the sample on ice until ready to run the PCR if desired.

Kit Storage

QuickBead Kits must be stored refrigerated. Note the shelf life on the kit box label.

Precautions and Notes

- The Kit is designed to be used with the protocol provided, which is optimized to work with the IMS technique developed by Ron Walcott *et al*. Deviation from the protocol may invalidate the results of the test.
- If recovery of viable bacteria via plating is desired, remove sodium azide from beads by rinsing three times with equivalent volume of 1X PBS before use.
- The use of a positive control wash (artificial or natural) is recommended to ensure the procedure has been correctly followed.
- Follow good lab practices to avoid cross contamination of samples.
- Preparation of 1X PBS, 1X PBS-Tween, and 10mM Tris-Tween:
  - 10X PBS stock (1L in distilled water):
    - 80g NaCl
    - 2g KCl
    - 11.5g Na_2HPO_4 (anhydrous)
    - 2g KH_2PO_4

- 1X PBS: dilute 100 mL stock with 900 mL water., final pH 7.4. Autoclave to sterilize.
- 1X PBS-Tween: add Tween 20 at a rate of 0.02% (200 µL Tween 20 to 1 L of 1X PBS following sterilization).
- 10mM Tris-Tween: 10mM Tris- 0.02% Tween pH 8.3
  - Tris-Base 1.21 g
    diH_2O 1 L
    Adjust pH to 8.3 then add Tween 20, 200 µL

  OR

  - Tris-Base 0.740 g
    Tris-HCl 0.614 g
    diH_2O 1 L
    Check pH (should be 8.3 at 25°C), then add Tween 20, 200 µL

- Materials and equipment needed for seed wash steps:
  - 10X PBS Stock
  - Orbital shaker
  - Flask or resealable plastic bag for soaking
  - Cheesecloth
  - Funnel
  - 1L collection flasks
  - High velocity centrifuge (8,000 rpm) for large seed wash samples
  - Pectinase, Sigma Cat # P2736 or equivalent
  - Whatman #1 filter paper
  - Screw-cap glass test tube, Pyrex No. 9825 or equivalent

- Materials and equipment needed for immunomagnetic separation:
  - Pipette(s) capable of delivering 1-10 mL and 20-250 µL
  - Buffer and solution materials: Tween, Tris-Base, diH_2O, Tris-HCl
  - End-over-end mixer (may use orbital shaker from seed washing instead)
  - Magnetic particle concentrator, Dynal MPC-L or equivalent
  - Plastic microcentrifuge tube
  - Centrifuge capable of holding microcentrifuge tube and a speed of 13,000 rpm
  - Heat block thermocycler

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