

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

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 in cell-buffer suspensions.

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 cattleyae*, *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.

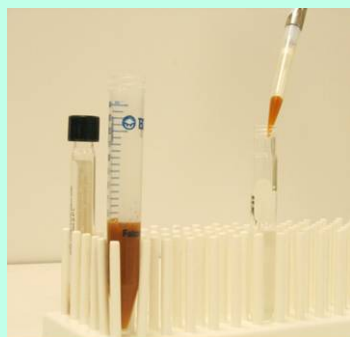
Preparation of the seed wash:

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.



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 the total μL of beads needed ($250\mu\text{L} \times$ number of tubes/subsamples to be tested) to an empty sterile tube. Apply the tube against the particulate concentrator (MPC); e.g. Dynal MPC-L from Invitrogen. Remove the storage buffer, and replace it with the same amount of IMS 1X PBS. Remove the tube from the MPC and resuspend the beads.
3. Pipette 0.25 mL of resuspended beads to the 6 mL of concentrated seed extract prepared in Step 7-Sample Preparation section. Incubate sample for 1 hour with end-over-end mixing at room temperature ($21\text{-}25^{\circ}\text{C}$, mix/agitate at ~ 30 rpm). Use an end-over-end mixer or the orbital shaker used in the seed wash steps.
4. Wash the incubated beads (remove and replace the buffer 4 times): Wash three times with 8 mL 1X PBS-Tween, then once with 8 mL of 10mM Tris-Tween. Remove and add buffer with a transfer pipette (e.g. Pasteur pipette) while beads have been attracted to the magnet for at least 1 minute. Between washes, resuspend the beads gently before returning the tube back to the MPC for the next round of wash (avoid agitating the beads too much).
5. For maximal concentration: After the last wash buffer removal, add $\sim 250 \mu\text{L}$ of 10mM Tris-Tween to the glass tube. Remove the tube from the MPC and mix gently to resuspend the beads. Then, using a transfer pipette, transfer all the beads to a 1.5 mL plastic microcentrifuge tube. Apply the micro tube against the MPC and remove the excess Tris-Tween. (If necessary, use this clear excess buffer to rewash the glass tube to recover any beads still remaining in there). Avoid creating too many bubbles.
6. Remove the microcentrifuge tube with just the beads from the MPC and carefully flush the beads to the bottom with 35-50 μL of 10mM Tris-Tween (use a fresh sterile Tris preparation). Give the tubes a quick spin (fast speed) to bring down any beads remaining attached to the walls of the microtube (*do so while the beads are wet; dry beads stick to the walls and are difficult to remove*).
7. To lyse bacteria and release DNA, heat the closed tube at 95°C in a heat block for 10 minutes. Next, centrifuge samples at 13,000 rpm for 30 seconds to collect the QuickBeads and cell debris at the bottom. Place the tubes back in the MPC to facilitate removal of DNA from the supernatant, leaving the beads behind. Remove from the tube a volume containing 5 μL less than the 35-50 μL volume used on step 6. Use this volume (extracted DNA) for PCR. Place the extracted DNA sample on ice until ready to run the test.

Notes: If Step 7 can not be completed on the same day, freeze the microtubes with the beads (Step 6). The following day, proceed with the heating, centrifuging and removal of supernatant for the PCR reaction (Step 7).

Make sure the temperature of the heating block is at 95°C , but below 100°C . Use a lab thermometer to verify it before starting. Temperatures over 100°C may destroy the bead's structure, releasing its content which could be detrimental to the PCR reaction.

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.
- 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 • 11.5g Na₂HPO₄ (anhydrous)
 - 2g KCl • 2g KH₂PO₄
 - 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
 - diH₂O 1 L
 - Adjust pH to 8.3 then add Tween 20, 200 µL
 - OR
 - { Tris-Base 0.740 g
 - Tris-HCl 0.614 g
 - diH₂O 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, diH₂O, 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
 - PCR equipment



**For Technical Support
Contact Us At:**

EnviroLogix
500 Riverside Industrial
Parkway
Portland, ME 04103-1486
USA
Tel: (207) 797-0300
Toll Free: 866-408-4597
Fax: (207) 797-7533

e-mail:

horticulture@envirologix.com

website:

www.envirologix.com



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