Xc

Immunoblot assay for *Xanthomonas campestris*
Catalog number: PBK 69000/0096

List of contents

Lot number	Item	/0096
_____	Anti-Xc antibody	0.200 ml
_____	Alkaline phosphatase enzyme conjugate	0.200 ml
_____	BCIP substrate solution	40 ml
_____	Positive control (if available)	1
	The above items should be stored at 4°C	
_____	Immunoblot membrane	1
_____	Dot blot bags	4
_____	Plastic wash container	1
_____	PBST wash buffer, 20X concentrate, 50 ml	2
_____	Nonfat dried milk	5 g
	The above items should be stored at room temperature	

Storage

Store all components at the recommended temperature to assure their full shelf life. Do not store prepared 1X buffers from day to day.

Safety

Prevent direct skin and eye contact with, or ingestion of, product components. Obtain medical attention in case of accidental ingestion of kit components. Always wash hands thoroughly after using this product.

If you have any questions about this product, please contact Agdia.

Preparing for the test

Note: Always wear latex gloves when handling the membrane.

Prepare 1X PBST buffer by diluting 20X PBST buffer according to package instructions. Agdia recommends preparing only as much 1X PBST buffer as is needed for one day.

Reconstitute lyophilized controls by adding 2 ml of distilled water to the vial. Once the controls are reconstituted they must be stored frozen (-20° C freezer or household freezer). Do not thaw until just before use.

The wash container included in this kit is large enough to process half of the total membrane. To process the entire membrane, you will need to obtain a container suitable to allow the membrane to lay flat with a small margin for processing. When using the provided container, the membrane will need to be cut in half prior to proceeding with the blocking step.

Otherwise, remove the number of columns you plan to process from the membrane prior to performing the assay.

Spot sap and air dry

To begin the test, spot 3 µl of undiluted plant sap onto each spot on the membrane. For your reference, record the locations of samples and controls.

If available, spot 3 µl of positive control and negative control onto individual spots. Be sure to include controls on partial membranes that will be individually processed. Label membrane sections using a permanent marker to ensure proper sample identification after processing.

Allow the membrane to air dry for 10 minutes. Make sure the membrane is completely dry before continuing.

Boil membrane

Submerge the membrane section in boiling water for 10 minutes

Prepare blocking solution

Prepare enough blocking solution to sufficiently cover the membrane. Half of a membrane will require about 5 ml of blocking solution, while an entire membrane will require about 10 ml of blocking solution.

Blocking solution consists of 2% nonfat dry milk in 1X PBST.

For example, if you are preparing 5 ml of blocking solution, you will need to mix 0.10 g of nonfat dry milk with 5 ml of 1X PBST buffer.

Add antibody

Place membrane and blocking solution in a dot blot bag.

Add anti-Xc antibody according to the dilution on the label.

For example, if you are using 5 ml of blocking solution, a dilution of 1:200 would require 25µl of anti-Xc antibody. Remove any air bubbles and seal the bag. Be sure that the membrane is flat and completely covered with solution while incubating.

Incubate membrane

Incubate on an orbital shaker at 150 rpm at room temperature for 45 minutes.

Wash membrane

Place the membrane in the wash container and wash 5 times for 2 minutes each with 1X PBST at room temperature.

Add enzyme conjugate

Place the membrane section in a fresh dot blot bag with the same volume of fresh blocking solution used before. Add enzyme conjugate according to the dilution on the label, then remove any air bubbles and seal the bag.

Remove the BCIP substrate solution from the refrigerator and allow it to warm to room temperature.

Incubate membrane

Incubate on an orbital shaker at 150 rpm at room temperature for 45 minutes.

Wash membrane

Place the sectioned membrane in the wash container and wash 5 times for 2 minutes each with 1X PBST at room temperature.

Add substrate

Pour 10 ml of the BCIP substrate solution over the membrane in the wash container. Protect the membrane from direct light while processing in the substrate solution.

Be sure the membrane is constantly submerged in BCIP substrate. Rock the container occasionally to distribute the substrate solution over the membrane.

Allow color to develop at room temperature for about 5-10 minutes, until the positive control is visibly colored.

Stop reaction

Stop reaction by washing the membrane with distilled water.

Interpret results

A purple spot or ring indicates a positive result. Be sure to check both sides of the membrane for the purple ring or spot.