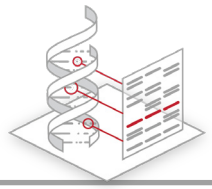
User Guide: ImmunoCapture Antibody for qPCR

ICA 20003 • *Potato virus Y* (PVY)



Intended Use:

The ImmunoCapture antibody for PVY is designed for use as the capture reagent in a single-tube qPCR system to simplify the sample purification process. Validation data for this product (see page 2) was derived from using qPCR primers and probe from Fageria and Singh *et al*.

SPECIFICITY: Binds all isolates of PVY. Does not bind with other potato pathogens.
SENSITIVITY: Dependent on the specific qPCR protocol the antibody is paired with.

Contents of Kit:

- Capture antibody

Not Included but Required:

- Carbonate coating buffer (ACC 00413)
- General Extract Buffer (GEB) (ACC 00955)
- PVY qPCR primers and probes
- PCR plate and cover
- qPCR reagents
- qPCR thermocyler

Kit Storage:

Kit components should be stored refrigerated (2 - 8 °C), unless specified otherwise below:

Test Preparation

1. Prepare a humid box by lining an airtight container with a wet paper towel.
2. Mix antibody thoroughly before use.

Prepare Capture Antibody

Scan for buffer formulations

1. Prepare the capture antibody (CAB) in a non-binding container, such as Agdia's sample cups (ACC 00960).
2. Dilute the thoroughly-mixed CAB, per the dilution on the label, in 1X carbonate coating buffer (see example). You will need 50 µL of diluted CAB per well; a full plate will need 5 mL.

   Example: (Wells Used 16 x 50 µL) ÷ 200† = 4 µL Capture Antibody
   †Bottle dilution

3. Thoroughly mix and pipette 50 µL of diluted CAB into each well of the provided PCR plate.
4. Incubate plate in the humid box overnight at 2 - 8 °C.
5. Coated plates should be used within 24 hours.

Prepare Samples

1. Sample symptomatic tissue if possible. Other plant parts may be tested, including asymptomatic tissue.
2. If compositing tubers, take 4 cores from 10 tubers each with a 3mm or 4mm biopsy punch.
3. At the time of testing, grind and dilute the samples at a 1:10 ratio with GEB.

   Example: 0.3 g plant tissue, extracted with 3 mL of GEB.

4. Empty coated plate contents and wash 3 times with 1X PBST.
5. Tap plate dry using lint-free paper towel.
6. Dispense 50 µL of the extracted samples into the plate.
7. Incubate plate in the humid box for either 4 hours at room temperature or overnight at 2 - 8 °C.

Prepare qPCR

1. Prepare the qPCR master mix.
2. Wash the sample from the plate 8 times using 1X PBST.
3. Tap plate dry using lint-free paper towel.
4. If needed, dry the plate in a 50 °C incubator until no liquid remains in the wells.
5. Dispense the qPCR reagents.
6. Cover the plate with optically clear film and run the qPCR.

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Inclusivity:

Isolates and Geographic Regions Detected:

PVY^{NE-11}	PVY-Alt ($PVY^{N:O}$)
PVY-HR1 (PVY^{Z}) (PVY^{NTN})	PVY-ID269 (PVY^{O-O5})
PVY-Mont (PVY^{N})	PVY-N1 (PVY^{N-Wi})
PVY-Oz (PVY^{O})	PVY-Poha2 (PVY^{C})
PVY-Poha6 (PVY^{C}) (PVY^{C-Poha})	PVY-Pondo4 (PVY^{261-4}) (PVY^{O})
PVY-PVY-AGA (PVY^{E}) ($PVY^{N/AST}$)	PVY-Tam15 (SA-N) (no serotype)[1]
PVY-Tam17 (SA-N) (N serotype)[1]	
[1]Isolate unable to systemically spread in potato (Green et. al.)	

Exclusivity:

Cross-reacts With:

None Known

Does Not Cross-react With:

Virus Name	Species Name
Andean potato latent virus (APLV)	Tymovirus latandigenum
Andean potato mottle virus (APMoV)	Comovirus andesense
Pepper mottle virus (PepMoV)	Potyvirus capsimaculae
Potato aucuba mosaic virus (PAMV)	Potexvirus marmoraucuba
Potato latent virus (PotLV)	Carlavirus latensolani
Potato leafroll virus (PLRV)	Polerovirus PLRV
Potato mop-top virus (PMTV)	Pomovirus solani
Potato virus A (PVA)	Potyvirus atuberosi
Potato virus M (PVM)	Carlavirus misolani
Potato virus S (PVS)	Carlavirus sigmasolani
Potato virus T (PVT)	Tepovirus tafsolani
Potato virus V (PVV)	Potyvirus vetuberosi
Potato virus X (PVX)	Potexvirus ecspotati
Tobacco etch virus (TEV)	Potyvirus nicotianainsculpentis
Tobacco rattle virus (TRV)	Tobravirus tabaci

Questions or Technical Support:

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