Barley yellow dwarf virus Reagent Set
Compound ELISA, Alkaline phosphatase label
Catalog number: SRA 26500 (BYDV-MAV), SRA 27500 (BYDV-PAV)

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<table>
<thead>
<tr>
<th>Lot number</th>
<th>Item</th>
<th>96 wells</th>
<th>500 wells</th>
<th>1000 wells</th>
<th>5000 wells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Capture antibody</td>
<td>0.150 ml</td>
<td>0.275 ml</td>
<td>0.525 ml</td>
<td>2.525 ml</td>
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<tr>
<td></td>
<td>Detection Antibody, bottle A</td>
<td>0.150 ml</td>
<td>0.275 ml</td>
<td>0.525 ml</td>
<td>2.525 ml</td>
</tr>
<tr>
<td></td>
<td>Alkaline phosphatase enzyme conjugate, bottle B</td>
<td>0.150 ml</td>
<td>0.275 ml</td>
<td>0.275 ml</td>
<td>2.525 ml</td>
</tr>
</tbody>
</table>

The above items should be stored at 4°C.

|            | 96-well microtiter plates, strip or solid                             | 1 strip  | 5 solid   | 10 solid   | 50 solid   |

The above items can be stored at room temperature.

Materials required, but not provided
- Carbonate Coating Buffer (formulation on page 6)
- PBST Wash Buffer (formulation on page 6)
- ECI Buffer (formulation on page 6)
- PNP Substrate Buffer (formulation on page 6)
- PNP Substrate Tablets (ACC 00404)
- General Extract Buffer (formulation on page 6)
- Buffer packs containing the above required items can be purchased from Agdia (ACC 00111)
- Distilled or purified water
- Paper towels
- Micropipette
- Micropipette tips
- Sample grinding device such as:
  - Agdia sample mesh bag (ACC 00930)
  - Agdia tissue homogenizer (ACC 00900)
  - Mortar and pestle
- Airtight container for incubations

Limitations

Expiration: This test should be used within 1 year of purchase.

Storage: Test results may be weak or the test may fail if storage instructions are not followed properly.

Buffers: Do not store 1X buffers from day to day. Buffers should be warmed to room temperature prior to use.

Dilutions: Read all labels carefully prior to preparing solutions to assure proper antibody concentrations. All antibody dilutions have been optimized for the greatest possible sensitivity and specificity based on available isolates and hosts. Using dilutions other than those listed can lead to potential false positives or false negatives.

Sample Extraction: Barley yellow dwarf viruses are limited to the phloem of the host plant. A grinding technique must be used that will disrupt the cells of the fibrous tissue. A mortar and pestle can be used if the sample is completely macerated. The use of liquid nitrogen or cellulase enzymes may provide a more efficient means of extraction in samples that are hard to grind, like grasses.

Sample Incubation: Barley yellow dwarf viruses are present in low concentrations in host plants. For this reason the sample incubation step must be done overnight in the refrigerator.
Precautions

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.

Intended Use

Agdia offers several tests for the detection of Barley yellow dwarf virus (BYDV). These test systems are useful for the detection of BYDV in leaves and roots of plants showing signs of infection. This instruction outlines the protocol for the BYDV-MAV (26500), and BYDV-PAV (27500) test systems.

The BYDV-MAV test system is specific to BYDV-MAV. The BYDV-PAV test system is specific to BYDV-PAV. These two test systems do not show cross-reaction to other related BYDV viruses.

Test Principle

The test systems for BYDV-MAV and BYDV-PAV are indirect DAS ELISA’s.

References


Technical Service

If you have any questions about using this kit, please contact Agdia, Inc. Monday – Friday by phone (574-264-2014 or 800-622-4342) or by email (info@agdia.com).
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Coat testwells of ELISA plate

1. Prepare humid box

   Prepare a humid box by lining an airtight container with a wet paper towel. Keeping testwells in a humid box during incubation will help prevent samples from evaporating.

   **Note:** All antibodies and enzyme conjugates should be prepared in a container made of a material such as polyethylene or glass that does not readily bind antibodies. Do not use polystyrene, polypropylene or polycarbonate.

2. Prepare capture antibody

   **Note:** 96 testwell reagents have a different dilution ratio than larger reagent sets. Please read label on capture antibody bottle for appropriate dilution ratio.

   The capture antibody is provided as a concentrated solution and must be diluted with carbonate coating buffer before use. The recommended antibody to buffer ratio is given on the label.

   Prepare the volume of carbonate coating buffer needed for the test. You will need 100 µl of carbonate coating buffer for each test well you are using. A full plate will require about 10 ml. Then, add the appropriate volume of concentrated capture antibody to the carbonate coating buffer at the dilution on the label.

   **Example 1:** If the dilution given on the bottle of concentrated capture antibody is 1:200, and you are preparing 10 ml of capture antibody solution, you should mix 10 ml of carbonate coating buffer with 50 µl of the concentrated capture antibody. Mix the prepared capture antibody solution thoroughly and use immediately.

   **Example 2:** If the dilution given on the bottle of concentrated capture antibody is 1:100, and you are preparing 10 ml of capture antibody solution, you should mix 10 ml of carbonate coating buffer with 100 µl of the concentrated capture antibody. Mix the prepared capture antibody solution thoroughly and use immediately.

3. Coat plate

   Pipette 100 µl of the prepared capture antibody solution into each well.

4. Incubate plate

   Incubate the plate in a humid box for 4 hours at room temperature or overnight in the refrigerator (4°C). Do not store coated plates longer than 24 hours. If long term storage is desired, contact Agdia about postcoat buffers.

5. Wash plate

   Empty the wells into a sink or waste container. Fill the wells completely with 1X PBST, and then quickly empty them again. Repeat 2 times.

   Hold the plate upside down and tap firmly on a folded paper towel to remove excess liquid.

   **Note:** Use freshly coated plates immediately.
Grind and dilute samples

When possible, select tissue showing symptoms for the test. Use Agdia’s general extract buffer (GEB) to grind and dilute samples.

*Barley yellow dwarf viruses* are limited to the phloem of the host plant. A grinding technique must be used that will disrupt the cells of the fibrous tissue. You can use Agdia’s sample mesh bags (ACC 00930), Agdia’s tissue homogenizer (ACC 00900), a mortar and pestle, or other grinding devices to grind samples. Make sure the sample is completely macerated. If using a mortar and pestle, wash and rinse it thoroughly between samples. The use of liquid nitrogen or cellulase enzymes may provide a more efficient means of extraction in samples that are hard to grind like grasses.

When you have extracted plant sap, dilute the sap into sample extraction buffer at a ratio of 1:10 (sap volume: buffer volume). Alternatively, grind plant tissue in extraction buffer at a 1:10 ratio (tissue weight: buffer volume).

You will need 100 µl of diluted sample extract per test well, plus an additional amount to assure easy dispensing. A convenient way to prepare this diluted sample is to measure 100 µl of undiluted sap into a small test tube, and then add 1 ml of extraction buffer.

Test Procedure

1. **Dispense samples**

   Following your loading diagram, dispense 100 µl of prepared sample into sample wells. Dispense 100 µl of positive control into positive control wells, and dispense 100 µl of extraction buffer into buffer wells.

2. **Incubate plate**

   Set the plate inside the humid box and incubate overnight in the refrigerator (4°C).

3. **Prepare enzyme conjugate**

   **Note:** Always prepare enzyme conjugate within 10 minutes before use.

   Both bottles of alkaline phosphatase enzyme conjugate and detection antibody (bottles A and B) are supplied as a concentrate and must be diluted with ECI buffer before use. The recommended conjugate to buffer ratio is given on each label. Dispense the appropriate volume of prepared ECI buffer into a dedicated container. You will need 100 µl of buffer for each testwell you are using. Then, add the alkaline phosphatase enzyme conjugate according to the dilution given on the labels.

   **Example 1:** If the dilution given on bottles A and B of concentrated detection antibody and alkaline phosphatase enzyme conjugate is 1:200, and you are preparing 10 ml of enzyme conjugate solution, you should first dispense 10 ml of ECI buffer. Then, add 50 µl from bottle A and 50 µl from bottle B to the ECI buffer.

   **Example 2:** If the dilution given on bottles A and B of concentrated detection antibody and alkaline phosphatase enzyme conjugate is 1:100, and you are preparing 10 ml of enzyme conjugate solution, you should first dispense 10 ml ECI buffer. Then, add 100 µl from bottle A and 100 µl from bottle B to the ECI buffer.

   After adding the reagents from bottles A and B, it is important to mix the enzyme conjugate solution well.
4. Wash plate

When the sample incubation is complete, wash the plate. Use a quick flipping motion to dump the wells into a sink or waste container without mixing the contents.

Fill all the wells completely with 1X PBST, and then quickly empty them again. Repeat 7 times.

After washing, hold the frame upside down and tap firmly on a folded paper towel to remove all droplets of wash buffer.

Inspect the test wells. All wells should be free of plant tissue. If tissue is present repeat the wash step and tap firmly on a paper towel.

5. Add enzyme conjugate

Dispense 100 µl of prepared enzyme conjugate per well.

6. Incubate plate

Incubate the plate in the humid box for 2 hours at room temperature.

7. Prepare PNP solution

Each PNP tablet (ACC 00404) will make 5 ml of PNP solution, at a concentration of 1 mg/ml, about enough for five 8-well strips.

About 15 minutes before the end of the above incubation step, measure 5 ml of room temperature 1X PNP buffer for each tablet you will be using. Then, without touching the tablets, add the PNP tablets to the buffer.

Note: Do not touch the PNP tablets or expose the PNP solution to strong light. Light or contamination could cause background color in negative wells.

8. Wash plate

As before, wash the plate 8 times with 1X PBST.

Inspect the wells looking for the presence of air bubbles. Tap firmly on the paper towel to remove remaining wash buffer and any air bubbles. If air bubbles are still present they may be broken with a clean pipette tip.

9. Add PNP solution

Dispense 100 µl of PNP solution per well.

10. Incubate plate

Incubate the plate for 60 minutes in a humid box. Plates should be protected from direct or intense light.

11. Evaluate results

Examine the wells by eye, or measure on a plate reader at 405 nm. Air bubbles which are present at the time of reading can alter results, if in the light path. Agdia recommends that bubbles be eliminated prior to reading.

Wells in which color develops indicate positive results. Wells in which there is no significant color development indicate negative result. Test results are valid only if positive control wells give a positive result and buffer wells remain colorless.

Results may be interpreted after more than 60 minutes of incubation as long as negative wells remain virtually clear.
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### Buffer formulations

**Carbonate Coating Buffer (1X)**
Dissolve in distilled water to 1000 ml:

- Sodium carbonate (anhydrous) 1.59 g
- Sodium bicarbonate 2.93 g
- Sodium azide 0.2 g

Adjust pH to 9.6. Store at 4° C.

**PBST Buffer (Wash Buffer) (1X)**
Dissolve in distilled water to 1000 ml:

- Sodium chloride 8.0 g
- Sodium phosphate, dibasic (anhydrous) 1.15 g
- Potassium phosphate, monobasic (anhydrous) 0.2 g
- Potassium chloride 0.2 g
- Tween-20 0.5 g

Adjust pH to 7.4

**ECI Buffer (1X)**
Add to 1000 ml 1X PBST:

- Bovine serum albumin (BSA) 2.0 g
- Polyvinylpyrrolidone (PVP) MW 24-40,000 20.0 g
- Sodium azide 0.2 g

Adjust pH to 7.4. Store at 4° C.

**PNP Buffer (1X)**
Dissolve in 800 ml distilled water:

- Magnesium chloride hexahydrate 0.1 g
- Sodium azide 0.2 g
- Diethanolamine 97.0 ml

Adjust pH to 9.8 with hydrochloric acid. Adjust final volume to 1000 ml with distilled water. Store at 4° C.

**General Extract Buffer (GEB 1X)**
Dissolve in 1000 ml 1X PBST:

- Sodium sulfite (anhydrous) 1.3 g
- Polyvinylpyrrolidone (PVP) MW 24-40,000 20.0 g
- Sodium azide 0.2 g
- Powdered egg (chicken) albumin, Grade II 2.0 g
- Tween-20 20.0 g

Adjust pH to 7.4. Store at 4° C.
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