User Guide: PathoScreen® Kit

Test Principle & Intended Use

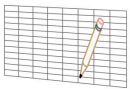
This product is intended for the qualitative detection of the target analyte via a direct, double antibody sandwich protocol known as DAS-ELISA. Upon successful completion of the test, samples containing the target analyte will turn yellow while negatives will remain colorless.

Handling Information

Antibodies and plates should be stored refrigerated (2 - 8 °C) between uses. All test materials should be warmed to room temperature (18 - 30 °C) before use. For materials provided please see the product webpage. Do not store 1X buffers for more than one day.

Safety

All components are non-hazardous except 5X PNP Buffer. Refer to component SDS for more information:
http://docs.agdia.com/DataSheets.aspx



Test Preparation

1. Record lot numbers of materials to be used in the test. Copy or print logsheet from the product webpage.
2. Prepare a humid box by lining an airtight container with a wet paper towel.
3. Mix both concentrated and diluted antibodies thoroughly before each use.

Sample Preparation

1. Sample symptomatic tissue if possible. Other plant parts may be tested, including asymptomatic tissue.
2. At the time of testing, grind and dilute the samples at a 1:10 ratio with General Extraction Buffer (GEB).

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

Positive and Negative Control Preparation

1. Use GEB to hydrate controls, according to label, at least five minutes before use.
2. Recap and mix thoroughly.

Test Procedure

1. Dispense 100 µL of the extracted samples, positive control, negative control, and extraction buffer into the plate following your logsheet.
2. Incubate plate in the humid box for either 2 hours at room temperature or overnight at 2 - 8 °C.

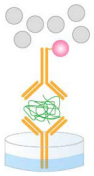
Prepare Enzyme Conjugate

1. Prepare the enzyme conjugate (ECA) in a non-binding container, such as Agdia's sample cups (ACC 00960).
2. Dilute the thoroughly-mixed ECA, per the dilution on the label, in 1X ECI buffer (see example). You will need 100 µL of diluted ECA per well; a full plate will need 10 mL.

   Example: (Wells Used 16 x 100 µL) ÷ 200[†] = 8 µL Enzyme Conjugate

   [†]Bottle dilution will be either 100 or 200
3. Wash the sample from the plate 8 times using 1X PBST.
4. Tap plate dry using lint-free paper towel.
5. Thoroughly mix and pipette 100 µL of diluted ECA into each testwell.
6. Incubate plate in the humid box for 2 hours at room temperature.

Agdia, Inc.
52642 County Road 1
Elkhart, IN 46514
574-264-2014 / 800-622-4342
www.agdia.com / info@agdia.com



Prepare Substrate

1. Add 1 PNP substrate tablet per 5 mL of 1X PNP substrate buffer into a dedicated container. You will need 100 µL of diluted PNP solution per well; a full plate will need 10 mL. Ensure tablets are dissolved before use. Keep prepared PNP solution in the dark prior to use.
2. Wash the ECA from the plate 8 times using 1X PBST.
3. Tap plate dry using lint-free paper towel.
4. Pipette 100 µL of dissolved PNP solution into each testwell.
5. Incubate, protected from light, for 1 hour at room temperature.

Evaluate Results

1. Examine wells by eye or measure with a spectrophotometer at 405 nm. Remove bubbles, if present, prior to reading.
2. Wells that develop color indicate positive results.
3. Wells in which there is no significant color development indicate negative results.
4. The test is valid only if known positive control turns yellow and known negative control remains colorless.

Warranty

Agdia reagents are warrantied for performance issues that arise from manufacturer defect. See product packaging for relevant expiration dates. Agdia's return policy can be found at www.agdia.com/customer-support/return-policy.

Additional Information

If you would like more information on how to run ELISA, please see Agdia's FAQ section, http://www.agdia.com/customer-support/frequent-questions-and-troubleshooting. For further documentation including this user guide, buffer formulations, and a logsheet, please see Agdia's specific product webpages. If you have problems with your ELISA or have technical questions, please contact techsupport@agdia.com.

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