User Guide: Compound Reagent Set

Test Principle & Intended Use

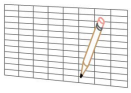
This product is intended for the qualitative detection of the target analyte via a direct, triple antibody sandwich protocol known as TAS-ELISA. Upon successful completion of the test, samples containing the target analyte will turn yellow while negatives will remain colorless.

Handling Information

Antibodies 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. The buffers necessary to run this assay can be purchased as buffer pack ACC 00111. Do not store 1X buffers for more than one day.

Safety

Components are non-hazardous. 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.

Prepare Capture Antibody

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 100 µL of diluted CAB per well; a full plate will need 10 mL.

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

[†]Bottle dilution will be either 100 or 200

3. Thoroughly mix and pipette 100 µL of diluted CAB into each testwell of the provided microtiter plate.
4. Incubate plate in the humid box for either 4 hours at room temperature (18 - 30 °C) or overnight at 2 - 8 °C.
5. Coated plates should be used within 24 hours. For longer storage recommendations please contact Agdia for postcoat buffer information.

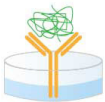
Sample Preparation

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. Empty contents and wash coated plate 3 times with 1X PBST.
2. Tap plate dry using lint-free paper towel.
3. Dispense 100 µL of the extracted samples, positive control, negative control, and extraction buffer into the plate following your logsheet.
4. Incubate plate in the humid box for either 2 hours at room temperature or overnight at 2 - 8 °C.

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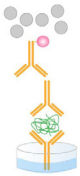
Prepare Detection Solution

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

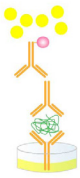
   Example: (Wells Used 16 x 100 µL) ÷ 200[†] = 8[‡] µL Bottle A and Bottle B
   [†]Bottle dilution will be either 100 or 200
   [‡]Add 8 µL of both Bottle A and Bottle B into 1X ECI
3. Wash the sample from the plate 8 times using 1X PBST.
4. Tap plate dry using lint-free paper towel.
5. Thoroughly mix and pipette 100 µL of the diluted detection solution into each testwell.
6. Incubate plate in the humid box for 2 hours at room temperature.

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 detection solution 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.

Limitations

Using microtiter plates other than the plate provided, especially low-bind or medium-bind, can greatly reduce sensitivity.

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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