User Guide: Qualitative Compound-ELISA Kit

PSP 73000 • neomycin phosphotransferase II (NPTII) • PEB1 / MRS-2 • Peroxidase

Test Principle, Intended Use and Limitations

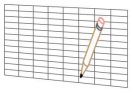
This product is intended for the qualitative detection of the target analyte via a direct, triple antibody sandwich protocol known as Compound-ELISA. Upon successful completion of the test, samples containing the target analyte will turn blue, due to the peroxidase enzyme label, while negatives will remain colorless. Visit the product webpage for information regarding host reactions, cross-reactions, alternate protocols, or other limitations.

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 user-prepared 1X buffers for more than one day.

Safety

Agdia recommends reading all relevant SDS sheets before using assay components: http://docs.agdia.com/datasheets.aspx.



Test Preparation

Scan for buffer instructions

1. Visit the product webpage to view buffer instructions, logsheet, and other documents.
2. Record lot numbers of materials to be used in the test using the logsheet.
3. Prepare a humid box by lining an airtight container with a wet paper towel.
4. Mix both concentrated and diluted antibodies thoroughly before each use.

Positive and Negative Control Preparation

1. Use 1X PEB1 extraction buffer to hydrate fresh controls, according to label, at least five minutes before use.
2. Recap and mix thoroughly.
3. Use of frozen or aliquoted controls comes with increased stability risks and may not match expected O.D. values.

Sample Preparation and Plate Loading

1. At the time of testing, grind and dilute the samples at a 1:10 ratio with 1X PEB1.

   Example: 0.3 g plant tissue, extracted with 3 mL of 1X PEB1.

2. Dispense 100 µL of the extracted samples, positive control, negative control, and 1X PEB1 into the provided antibody coated microtiter plate following your logsheet.
3. Incubate plate in the humid box for either 2 hours at room temperature or overnight at 2 - 8 °C.
4. For greater sensitivity, incubate the sample for 2 hours. An overnight incubation can result in a reduced limit of detection.

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 MRS-2 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) ÷ 100† = 16‡ µL Bottle A and Bottle B
   †Bottle dilution will be either 100 or 200
   ‡Add 16 µL of both Bottle A and Bottle B into 1X MRS-2

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.

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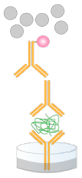
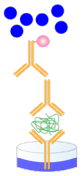


Prepare Substrate

1. TMB is a ready to use solution. Keep in the dark until 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 TMB into each testwell.
5. Incubate, protected from light, for 15 minutes at room temperature.

Interpreting Results

1. Visually inspect wells and remove bubbles, if present. Measure O.D. values with a spectrophotometer at 650 nm.
2. **Optional:** Pipette 100 µL of 1N HCl stop solution into each testwell. Measure O.D. values with a spectrophotometer at 450 nm up to 1 hour after addition.
3. The test is valid if the positive and negative control O.D. results meet expected values (see Certificate of Analysis).
4. Sample interpretations should be performed on a case-by-case basis. Plant tissue interactions with ELISAs can vary greatly between plant species and even varieties. Certain tissues can cause an elevated or higher than normal O.D. value. In this case, a sample(s) of the same species or variety is needed to determine the negative average.



5. Generally, positive and negative thresholds can be determined by using 2 times the negative average. Any samples with an O.D. value higher than 2 times the negative average are positive, and samples with an O.D. value below 2 times the negative average are negative. An alternative method for threshold calculations is the negative average plus 3 times the standard deviation of the negative sample set.

Method 1	*Negative Avg.*	*0.065*	*2 x Negative Avg.*	*0.130*
	Sample 1	*0.255 (Positive)*	*Sample 2*	*0.115 (Negative)*

Method 2	*Negative Avg.*	*0.065*	*Std. Dev.*	*0.030*	*Negative Avg. + 3 x Std. Dev.*	*0.155*
	Sample 1	*0.255 (Positive)*	*Sample 2*	*0.115 (Negative)*		

6. Positive O.D. values indicate the presence of the target protein (or in some cases, a closely related protein).

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. For answers to your technical questions, please contact us at techsupport@agdia.com.

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