User Guide: Quantitative 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 quantitative detection of the target analyte via a direct, triple antibody sandwich protocol known as Compound-ELISA. Upon successful completion of the test, the NPTII protein standard will yield a linear standard curve with an analytical range of between 1 ng/mL and 18 ng/mL which will allow for the quantification of NPTII protein content in the samples. Visit the product webpage for information regarding host reactions, cross-reactions, alternate protocols, or other limitations. **The NPTII protein standard, LST 73000, can be purchased separately and is required for quantitative use.**

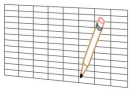
Handling Information

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.

Scan for buffer instructions



Test Preparation

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.

Negative Control Preparation

1. The positive control provided with the kit is for qualitative purposes only
2. Use 1X PEB1 extraction buffer to hydrate fresh negative controls, according to label, at least five minutes before use.
3. Recap and mix thoroughly.
4. Use of frozen or aliquoted controls comes with increased stability risks and may not match expected O.D. values.

Protein Standard Preparation

1. The positive control provided with the kit is for qualitative purposes only
2. Rehydrate the NPTII protein standard with 1 mL of 20 mM Tris pH 8.0 and mix well.
3. Let the NPTII protein standard sit for 1 hour and mix well again.
4. Dispense aliquots sufficient for one use in a container appropriate for storage at -80 °C.
5. Prepare a 5 point standard curve beginning at 18 ng/mL.
6. Dilute the NPTII protein standard in negative host matrix.
7. A 1:2 dilution is suggested for the standard curve with concentrations of 18, 9, 4.5, 2.25, and 1.125 ng/mL used.
8. Include both a buffer only and matrix only testwells for determination of limit of detection/quantitation.

Sample Preparation and Plate Loading

1. For quantitative analysis, it is important that the concentration of NPTII in the samples be within the range of the assay.
2. It is recommended to perform a serial dilution of one or more representative samples the first time testing to determine the typical concentration of NPTII found within your samples.
3. Start with a dilution ratio of 1:5 and serial dilute the extract in 1X PEB1 to achieve 1:10, 1:20, and 1:40 ratios
4. At the time of testing, grind and dilute the samples at the dilutions above or the dilution determined to have the correct typical concentration of NPTII in 1X PEB1.

   Example (1:10): 0.3 g plant tissue, extracted with 3 mL of 1X PEB1.

5. Dispense 100 µL of each NPTII protein standard into each dilutions testwell(s).
6. Dispense 100 µL of the extracted samples, negative control, and 1X PEB1 into the provided antibody coated microtiter plate following your logsheet.
7. Incubate plate in the humid box for either 2 hours at room temperature or overnight at 2 - 8 °C.
8. For greater sensitivity, incubate the sample for 2 hours. An overnight incubation can result in a reduced limit of detection.

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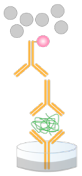
Prepare Detection Solution

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 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.

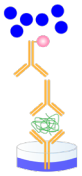
Prepare Substrate



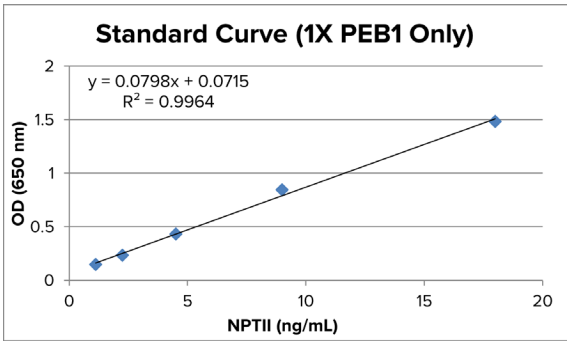
1. TMB is a ready to use solution. Keep in the dark until use.
2. Wash the detection solution from the plate 8 times using 1X PBST.
3. Tap plate dry using lint-free paper towel.
4. Pipette 100 µL of TMB into each testwell.
5. Incubate, protected from light, for 15 minutes at room temperature.

Interpreting Results

1. Visually inspect wells and remove bubbles, if present. Measure O.D. values with a spectrophotometer at 650 nm.
2. Using the O.D. values from the NPTII protein standard wells construct a standard curve by plotting O.D. values versus the NPTII protein standard concentrations.
3. The standard curve should be linear. If the curve is not linear, contact Agdia for assistance.
4. Sample NPTII concentrations can be calculated by the following equation:
   concentration = (sample O.D. value - y-intercept) / slope of standard curve



   Example: Standard curve: y = 0.0798x + 0.0715, R^2 = 0.9964
   Sample O.D. = 0.500
   Concentration = (0.500 - 0.0715) / 0.0798 = 5.37 ng / mL
5. The graph to the right depicts typical performance of the NPTII protein standard serially diluted in 1X PEB1. Different host matrices could have an effect on the curve.



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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