

User Guide: Nucleic Acid Hybridization Kit

NAK 63900 • *Avocado sunblotch viroid* (ASBVd) • Ames buffer



Test Principle, Intended Use and Limitations

This product is intended for the qualitative detection of the target analyte via a nucleic acid hybridization assay protocol. The spotted membrane is submitted to Agdia Testing Services where it is processed and results are reported back to you. Visit the product webpage for information regarding host reactions, cross-reactions, alternate protocols, or other limitations.

Handling Information

Membrane kit components should be stored at room temperature (18 - 30 °C). For materials provided please see the product webpage. Gloves should be worn when handling the membrane and when using chloroform. Chloroform steps should be performed with protective ventilation.

Safety

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

Test Preparation

1. Visit the product webpage to view the required components that are not included.
2. Record membrane number on the logsheet.
3. Do not spot any samples to the left of the solid line on the membrane. This column is reserved for Agdia controls.
4. Do not cut and submit part of a membrane. Only entire membranes will be accepted for processing. A partially spotted membrane may be held for completion of spotting later.

Sample Collection

1. Agdia recommends that individual leaves be tested from more than one location on a tree, as viroid titer is not even throughout the tree (Figure 1).
2. Collect samples from the 3rd or 4th leaf from the terminal bud. Leaves should be green and fully open (Figure 2).
3. Compositing is **not** recommended as this could lead to false negatives when the sample has a minimal viroid concentration. Only one leaf should be extracted per spot on the hybridization membrane.

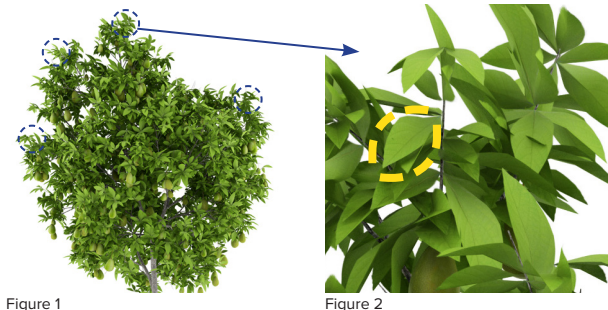


Figure 1

Figure 2

Sample Preparation

1. Grind the samples using a leaf press, mortar and pestle, or sample extraction bags to generate 100 µL of plant sap.
Note: Thoroughly grind the samples to release the chloroplasts.

Example: 0.1 g of tissue ground with 100 µL of water. (If this does not produce 100 µL of plant sap, add water to reach 100 µL.)

2. Dilute the samples at a 1:1.5 ratio with Ames buffer.

Example: 100 µL plant sap, extracted with 150 µL of Ames buffer.

Sample Incubation

1. Incubate the samples at 34 - 40 °C for 15 minutes.
2. After 15 minutes, add an equal volume of research grade chloroform to each sample. Mix thoroughly by shaking, vortexing, or inverting.
3. Briefly centrifuge the samples to separate the aqueous (top) and chloroform (bottom) layers. The layers must be clearly separated before proceeding.
- 3a. Alternatively, samples can be placed in the refrigerator (2 - 8 °C) overnight.



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Extract Spotting

1. For your reference, complete a logsheet to show the locations of your samples. It is not necessary to include a copy of the diagram when returning the membrane to Agdia.
2. Wearing gloves, open the clear plastic sleeve protecting the membrane and fold it under for spotting. Do not remove the membrane from the sleeve.
Note: Avoid touching the membrane as it can cause non-specific marks which can obstruct sample spots on the final film.
3. For each sample, pipette 2.0 µL of extract from the aqueous layer 4 mm to the right of the selected dots on the membrane – **do not pipette the extract directly on the dot.**
Note: Spotting material from the interphase, the region between the top and bottom phases, can cause false positive or questionable results.
4. Allow the membrane to air dry, then close the jacket and replace the membrane in its protective folder.
5. Store all sample extracts at 2 - 8 °C until results are provided. If you need to retest any samples, you can use the stored extracts or extract fresh material.

Membrane Submission to Agdia Testing Services

Domestic Customers

1. Complete Membrane Submission Form A (included with your kit).
2. Return it to Agdia Testing Services with your spotted membrane by the expiration date on the plastic sleeve.
Note: Not completing and including this form could delay your results.

International Customers

IMPORTANT: Customers from outside the United States must submit their membranes to Agdia using a special NAK permit packet. This packet facilitates the transfer of your membranes from your country of origin, through a USDA APHIS inspection station, and finally to Agdia. This process is required under U.S. law. Permit packets can be ordered from Agdia for a fee. If Red/White labels have expired, please contact Agdia for replacement. Any membranes submitted to Agdia without using the permit packet cannot be processed and will be destroyed upon receipt.

1. Complete Membrane Submission Form B (included with your kit).
2. Return it to Agdia Testing Services with your spotted membrane by the expiration date on the plastic sleeve.
Note: Not completing and including this form could delay your results.

Warranty

Agdia reagents are warranted for performance issues that arise from manufacturer defect. See product packaging for relevant expiration dates. Agdia does not guarantee results for membranes submitted after the expiration date. Agdia's return policy can be found at www.agdia.com/customer-support/return-policy.

Additional Information

For further documentation, including this user guide and a membrane submission form, please see Agdia's specific product webpages. For answers to your technical questions, please contact us at techsupport@agdia.com.



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Logsheet for Nucleic Acid Hybridization Assay

Membrane Number: _____ Date: _____ Samples Spotted By: _____

	Agdia Controls	1	2	3	4	5	6	7	8	9	10	11	12
A													
B													
C													
D													
E													
F													
G													
H													

Note: The shaded column is reserved for Agdia Controls. Do not apply samples to these spots.



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