

## List of contents

Lot number	Item	Quantity
_____	Nylon membrane	1
_____	Ames buffer	60 mL
_____	Membrane Submission Form	1

*The above items should be stored at room temperature (18 - 30 °C).*

## Not included but required

- NAK Permit Packet (catalog NAK 07500) for Foreign Shipments only
- Microcentrifuge tubes
- Research grade chloroform
- Micropipette
- Micropipette tips
- Gloves
- Forceps (optional for handling membrane)
- Fume hood
- Microcentrifuge (optional)

## Preparing for the test

Familiarize yourself with the kit components. Check that all components are present in the kit.

Gloves should be worn when handling membrane and when using chloroform.

All steps working with chloroform should be done in a fume hood.

Store membrane in the protective folder in a cool, dry place until ready to use. Refrain from handling it before and after spotting the samples.

A column to the left, indicated by a line, is reserved for our controls. Please do not spot anything on the positions within this column.

Ames buffer comes ready to use and does not need to be diluted. Store Ames buffer at room temperature, as particulates may form if the buffer is too cold. If this occurs, place the bottle briefly in a warm water bath until they are back in solution.

If you have any questions about using this kit, please contact Agdia, Inc. by phone (574-264-2014 or 800-622-4342) or by email ([info@agdia.com](mailto:info@agdia.com)).

## Reference

Podleckis, E.V., Hammond, R.W., Hurtt, S.S., and Hadidi, A. (1993) Chemiluminescent detection of potato and pome fruit viroids by digoxigenin-labeled dot blot and tissue blot hybridization. *J. Virol. Methods* 43, 147-158.

## Prepare samples

It is necessary to squeeze sap or grind tissue from the plant sample and dilute with Ames buffer. A leaf press or grinder can be used to express sap. Samples can also be ground using a mortar and pestle or sample extraction bag. Typically, samples should be diluted with Ames buffer in a microcentrifuge tube at a sample to buffer ratio of 1:1.5. For example, mix 100 µL of sap with 150 µL of Ames buffer.

- If sap cannot be obtained, grind 0.1 g of dry tissue with 150 µL of Ames buffer.
- For tubers, mix 150 µL of sap with 100 µL Ames buffer.

## Incubate samples

Cover the microcentrifuge tubes and incubate at 34 - 40 °C for 15 minutes.

Wearing gloves and working in a fume hood, add an equal volume of research grade chloroform (not included) to each tube. Close caps tightly, then mix contents thoroughly by shaking, vortexing, or inverting.

It is recommended to briefly centrifuge tubes to separate the contents into aqueous (top) and chloroform (bottom) layers. Alternatively, tubes can be placed in a refrigerator (2 - 8 °C) overnight. The layers must be clearly separated before proceeding.

## Spot extracts

For your reference, complete a loading diagram to show the locations of your samples. It is not necessary to include a copy of the diagram when returning the membrane to Agdia.

Wearing gloves, open the clear plastic sleeve protecting the membrane and fold it under for spotting. Do not remove the membrane from the sleeve. Avoid touching the membrane as it can cause non-specific marks which can obstruct sample spots on the final film.

Following the loading diagram; pipette 2.0 µL of extract from the aqueous layer of sample tubes onto selected dots on the membrane. Spotting material from the interphase, the region between the top and bottom phases, can cause false positive or questionable results. Retesting would be recommended for that sample. If spots begin to touch each other, reduce the volume pipetted.

Allow the membrane to air dry, then close the jacket and replace the membrane in its protective folder.

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.

## Submitting the Membrane Back to Agdia

### U.S. Customers

Complete **Membrane Submission Form A** (included with your kit). Return it to Agdia with your spotted membrane by the expiration date on the plastic sleeve. 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.

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

**Please Note:**

- 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.
- Agdia does not guarantee results for membranes submitted after the expiration date.

**Ames buffer (1X)**

Ames buffer is a standard part of the kit. It comes ready to use and should not be diluted. The formulation provided is for reference.

Distilled water	160.0 mL
Sodium chloride	11.7 g
Magnesium chloride	0.4 g
Sodium acetate	8.21 g
Ethanol	40.0 mL
Sodium dodecyl sulfate (SDS)	6.0 g

Add the sodium chloride to the water. After it has dissolved, add the magnesium chloride and sodium acetate. After all salts have dissolved, add the ethanol, then add SDS. Adjust pH to 6.0 with hydrochloric acid or sodium hydroxide.

Loading diagram 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 our controls.  
Do not apply samples to these spots.**