

## Virus Overlay Assay (Far-Western Blotting)

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**[Abstract]** Virus overlay assay is a method to detect protein-protein interaction *in vitro*. We performed the virus overlay assay to identify the receptor proteins interacting with the infectious spleen and kidney necrosis virus (ISKNV).

### **Materials and Reagents**

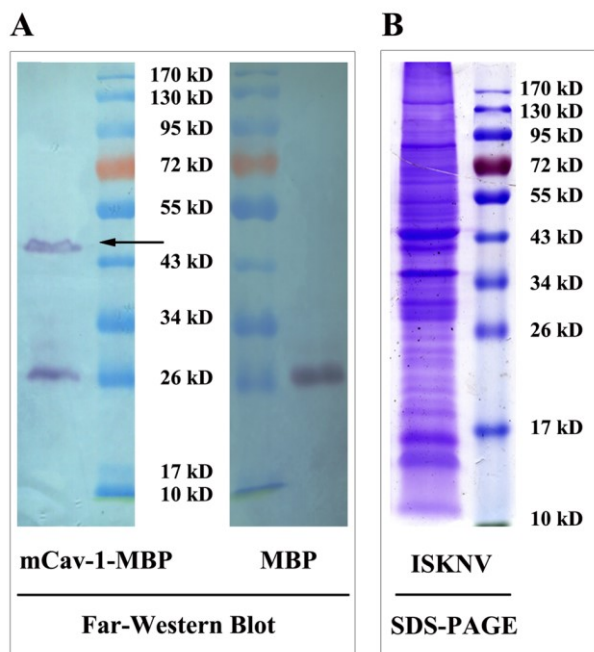
1. Purified ISKNV (was purified in our laboratory)
2. 12% SDS-PAGE gel
3. PVDF membrane (Whatman, catalog number: 10485290)
4. Coomassie brilliant blue G-250 (Beyotime Institute of Biotechnology, catalog number: P0017B)
5. Tris-HCl (pH 7.4)
6. EDTA
7. Tween 20
8. Guanidine hydrochloride (Sigma-Aldrich, catalog number: G4505)
9. Nonfat milk powder
10. Maltose-binding protein (MBP) or MBP-mCav-1 protein (these proteins were made in our laboratory)
11. Anti-MBP antibodies (Sigma-Aldrich, catalog number: M1321)
12. Horse radish peroxidase (HRP)-conjugated goat anti-mouse secondary antibodies (Invitrogen, catalog number: 626520)
13. HRP substrate solution (Beyotime Institute of Biotechnology, catalog number: P0209)
14. BCA protein assay kit (Thermo Fisher Scientific, catalog number: 23225)
15. Renaturing buffer (see Recipes)
16. Tris Buffered Saline and Tween 20 (TBST) (see Recipes)

### **Equipment**

1. Electrophoresis equipment
2. Bio-Rad blotting equipment (Bio-Rad Laboratories, model: A101441)

## **Procedure**

1. The protein concentrations of the purified virus are determined using the BCA protein assay kit. 100 micrograms of the sample are boiled in 2x SDS-PAGE sample loading buffer for 5 min.
2. 30  $\mu$ l (100 micrograms) denatured product is resolved in two parallel 12% SDS-PAGE gels.
3. After electrophoresis, the viral structural proteins in one gel are transferred to a PVDF membrane by electroblotting using the Bio-Rad wet transfer Blotter (50 V for 2 h), whereas the other gel is stained with Coomassie brilliant blue G-250 for a parallel experiment.
4. The membrane is washed twice in TBST at room temperature (RT) under constant rotation for 5 min each.
5. The blots are then blocked overnight in renaturing buffer at 4 °C, and then incubated with 10  $\mu$ g/ml MBP or MBP-mCav-1 protein for 2 h at RT.
6. The membrane is incubated with anti-MBP antibodies (1:2,000 dilution) in TBST for 2 h at RT after washing three times in TBST under constant rotation for 10 min each.
7. The membrane is washed as described in step 4, and the antigen-antibody complexes are then detected using HRP-conjugated goat anti-mouse secondary antibodies (1:5,000 dilution) in TBST for 1 h under constant rotation.
8. The membrane is washed as described in step 4, the immobilized conjugates on the membrane are subsequently visualized using an HRP substrate solution. When the member reaches desired intensity, pour off the HRP substrate solution and rinse in several changes of distilled water. Incubate in ddH<sub>2</sub>O for ~20 min, see an example of the results in Figure 1.



**Figure 1. A. Far-Western blot analysis; B. Purified ISKNV particles (100 µg) were isolated using SDS-PAGE, and then stained with Coomassie brilliant blue R-250.**

### Recipes

1. Renaturing buffer
  - 20 mM Tris-HCl
  - 1 mM EDTA
  - 0.5 mol/L NaCl
  - 0.05% Tween 20
  - 1 M guanidine hydrochloride
  - 5% nonfat milk powder
2. TBST (1 L)
 

NaCl	8 g
KCl	0.2 g
Tris base	3 g

Mix in ~ 800 ml dH<sub>2</sub>O, adjust pH to 7.4 with HCl, then adjust volume to 1 L

Autoclave

After cooling, add 1 ml Tween 20

### Acknowledgments

This protocol is adapted from Kikkert *et al.* (1998).

**References**

1. Kikkert, M., Meurs, C., van de Wetering, F., Dorfmuller, S., Peters, D., Kormelink, R. and Goldbach, R. (1998). [Binding of tomato spotted wilt virus to a 94-kDa thrips protein](#). *Phytopathology* 88(1): 63-69.