Immunoblot Screening of Chlorella Virus Plaques

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Abstract

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Guidelines

Materials:

1. Glass petri plates of NC64A chlorella lawns with virus plaques (use plates that have been incubating for 3-4 days)
2. Nitrocellulose circles
3. Tris buffered saline + Tween 20 (TBST, 1X): 10 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.05% Tween 20
4. Blocking solution: 1% BSA in 1X TBST
5. Alkaline phosphatase buffer (AP, 1X): 100 mM Tris-HCl, pH 9.5, 100 mM NaCl, 5 mM $MgCl_2$
6. Reaction stop/storage buffer (1X): 20 mM Tris-HCl, pH 8.0, 5 mM EDTA
7. Primary (1°) antiserum: Chlorella virus antiserum, produced in rabbits
8. Secondary (2°) antiserum: Anti-rabbit IgG alkaline phosphatase conjugate
9. NBT substrate: Nitro blue tetrazolium
10. BCIP substrate: 5-bromo-4-chloro-3-indolyl phosphate
11. Glass petri dishes
12. Rotary shaker
13. Reaction mixture: 0.33 mg/mL NBT + 0.17 mg/mL BCIP in 1X AP buffer. Dissolve the BCIP in 500 µL of dimethylformamide, then add dropwise to the NBT dissolved in the AP buffer

Protocol

Step 1.
Take the desired petri plates of virus plaques on chlorella lawns and chill in the cold room for 30-60 min prior to use.

DURATION
01:00:00

Step 2.
Overlay the plates with the nitrocellulose circles.

Step 3.
Allow the nitrocellulose to lay on the agar surface for 15 min.

DURATION

00:15:00

NOTES

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Take care that there are no air bubbles between the agar surface and the nitrocellulose.

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If a double transfer of the plates is desired, replace the first nitrocellulose filter with a second filter and allow the second to lay on the agar surface for 30 min.

Step 4.

Allow the filters to air dry.

Step 5.

Add 7.5-8.0 mL of the blocking solution to each filter in a glass petri plate and incubate for 30 min with gentle shaking (approximately 100 rpm on the rotary shaker).

DURATION

00:30:00

Step 6.

Decant the blocking solution from the petri plates.

Step 7.

Add 7.5-8.0 mL of a 1/600 dilution of the 1° antiserum in 1X TBST to each filter and incubate for 30 min, with gentle shaking.

DURATION

00:30:00

Step 8.

Decant the antiserum from the petri plates.

Step 9.

Wash each filter 3X with 8.0 mL of 1X TBST for 10 min, with gentle shaking.

DURATION

00:10:00

Step 10.

Decant the TBST solution from the petri plates.

Step 11.

Add 7.5-8.0 ml of a 1/7500 dilution of the 2° antiserum in 1X TBST to each of the filters and incubate for 30 min, with gentle shaking.

DURATION

00:30:00

Step 12.

Decant the antiserum solution from the petri plates.

Step 13.

Wash each filter 3X with 8.0 mL of 1X TBST for 10 min, with gentle shaking.

DURATION

00:10:00

Step 14.

Decant the TBST solution from the petri plates.

Step 15.

Add 7.5-8.0 mL of the reaction mixture to each of the filters, cover with aluminum foil and incubate with gentle shaking.

Step 16.
When the color development is sufficient, decant the reaction mixture from the petri plates.

Step 17.
Add 8.0 mL of the stop/storage buffer to each filter and incubate for 30 min, with gentle shaking.

DURATION
00:30:00

Step 18.
Place the filters into a glass pyrex baking dish with the stop/storage buffer and incubate for 30 min at room temperature.

DURATION
00:30:00

Step 19.
Place in the cold room overnight.

DURATION
18:00:00

Step 20.
Remove the filters from the stop/storage buffer and allow to air dry on paper towels or filter papers.

Step 21.
Store the filters in small plastic sealed bags.