Tissue Print Immunoblot Analysis

1) Solutions

PBS: 10 mM NaH_2PO_4-Na_2HPO_4 (pH7.2), 0.9% (w/v) NaCl

PBS-BSA: PBS, 0.3% (w/v) BSA (Fatty acid free: Sigma; A6003)

TBST: 100 mM Tris-HCl (pH 7.5), 0.1% (v/v) Tween-20, 0.9% (w/v) NaCl

TBST-Milk: TBST, 5% (w/v) non-fat dry milk (Dainippon Sumitomo Pharma; BlockAce)

Normal goat serum: (Vector Lab. Inc.; S-1000)

Affinity-purified rabbit primary antibody: Dilute with PBS-BSA, 0.05% (w/v) NaN_3.

ABC (Avidin:Biotinylated enzyme Complex) Kit:
VECTASTAIN *Elite* ABC Rabbit IgG Kit (Vector; PK-6101)

- **Goat biotinylated anti-rabbit IgG solution**: 150 µl of normal goat serum, 50 µl of goat biotinylated anti-rabbit IgG in 10 mL of TBST
- **ABC solution**: 200 µl of avidinDH, 200 µl of biotinylated horseradish peroxidase (HRP) in 10 mL of TBST

  *ABC solution must be prepared and stand for 30 minutes before use.

DAB (3,3'-diaminobenzidine) solution: 100 mM Tris-HCl (pH 7.5), 0.1% (w/v) DAB, 0.03% (v/v) H_2O_2, 0.04% (w/v) $NiCl_2$

2) Tissue-printing on the nitrocellulose membrane

Draw square grids (1 X 1 cm) on the nitrocellulose membrane (rectangle 2 X 4 cm, Scheleicher & Schuell; Type BA-85) with a pencile.
Immerse the membrane in PBS and shake it gently for 30 minutes.

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Place the membrane on the filter paper (Advantec Toyo; No.5A) and air dry it for about 5 to 10 minutes. * Not dry the membrane completely.

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Prepare a blotting apparatus (Fig. 1).

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Cut out one-mm thickness of the transverse section from the plant organ.

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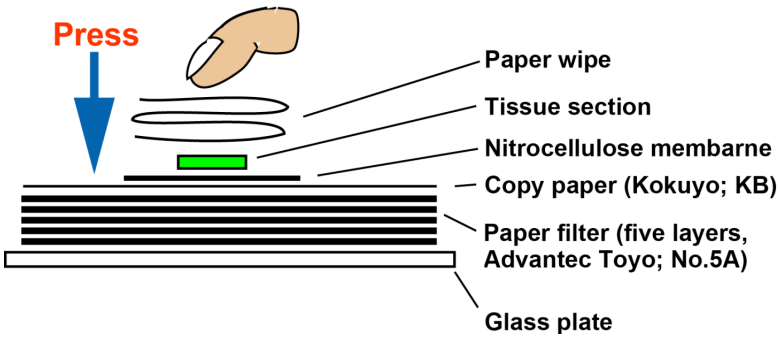
Fig. 1



Put the cross-section onto the semi-dried membrane.

Overlay the section with four layers of paper wipes (Crecia; kimwipe S-200) and press for 20 seconds by acupressure.

Remove the section from the membrane.

3) Immunodetection using the ABC method

Shake the tissue-printed membarane gently in TBST for 30 minutes at room temperature.

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<Blocking>

Shake the membrane gently in TBST-Milk for 1 hour at room temperature.

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<Primary antibody stain>

Shake the membrane gently in the primary antibody solution at 37℃ over-night.

Wash the membrane in TBST for 20 minutes by gentle shaking (three times).

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<Secondary antibody stain>

Shake the membrane gently in the goat biotinylated anti-rabbit IgG solution for 1 hour at room temperature.

Wash the membrane in TBST for 20 minutes by gentle shaking (three times).

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<ABC Stain>

Shake the membrane gently in the ABC solution for 1 hour at room temperature.

Wash the membrane in TBST for 5 minutes by gentle shaking (three times).

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<Detection>

Incubate the membrane in the DAB solution until observing clear dark blue staining.

Stop coloring by rinsing the membrane with water.

Observe immuno-staining using the stereoscopic microscope.