

Immunohistochemical Whole Mounted Tissue Protocol for Antibodies for Phytohormones
(Trans-Zeatin, Indole-3 Acetic Acid and N6-(Δ^2 -isopentenyl) adenine-9-riboside) co-localized
with DAPI within *Eurosta solidaginis*

Dissection:

* Check if animal is alive by poking and seeing movement, color should be semi-opaque/white/light yellow. Dark yellow and no movement means sample is not viable.

1. Wash off detritus from larvae in PBS before dissection.
2. Dissect larvae by holding denticles and then slicing anterior to posterior with microblade.
3. Remove tissues from body wall by prying them away with minuten.
4. Place in PBS while collecting other samples in 2ml centrifuge tubes filled with 25 μ l of PBS.

Fixation:

1. Place whole mounted body part (gut, salivary glands and body wall) in cold fixative solution of 3% paraformaldehyde in PBS for 30 minutes.
2. Place samples in fresh fixative solution on ice for 2.5 hours.
3. Wash samples 3 times in PBS, 45 mins each wash and then store in PBS at 4°C overnight.

Antibody Staining:

1. Wash samples with blocking buffer (1%BSA) 3 times, 10 minutes each wash.
2. Wash 30 minutes in PBT (PBS/ Triton .1%)
3. Transfer samples to .05 ml microcentrifuge tubes. Incubate samples in the primary antibody solution (1:250 anti-tZ antibody or 1:250 anti-iAA or 1:250 anti-iPR in 1% BSA) 48 hrs at 4° C.

Antibody Staining Cont'd:

4. Allow samples to come to room temperature on the bench, then wash with PBS five times, 10 minutes each wash.
5. Add the secondary antibody (goat anti-rabbit *FITC conjugated diluted 1:1000 in PBS solution) to the samples and incubated for 18 hours in the dark at 4C.

Antibody Staining Cont'd:

6. Wash 5 times for 10 min each in PBSBT
7. Transfer glands to slide, VECTASHIELD®, cover with nail polish

For co-localization + DAPI:

8. Dilute the DAPI stock solution to 1:1000 in PBS. Add approximately 300 µL of this dilute DAPI staining solution to the microcentrifuge tube.
9. Incubate for 8 minutes.
10. Wash in PBS 3 times for 10 mins each wash.

11. Transfer tissue sample to slide, add a drop of VECTASHIELD® as mounting agent, seal with nail polish.

Reagents:

- Phosphate Buffered Saline (PBS x1M)
- 3% paraformaldehyde in Phosphate Buffered Saline (PBS)
- 1% Bovine Serum Albumin
- PBT (PBS + .1% triton)
- PBSB (PBS + 1% BSA)
- PBSBT (PBS + .1% triton and 1%BSA)
- 1/250 anti-*tZ* antibody or 1/250 anti-*iPA* or 1/250 anti-*IAA* in PBSB.
- DAPI (4',6-diamidino-2-phenylindole)
- VECTASHIELD®