Technical Note

Immunohistochemistry for Insect Histology and Pathology Diagnosis

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Posted Date: 17 July 2024

doi: 10.20944/preprints202407.1354.v1

Keywords: insect morphology; IHC; TUNEL; HE

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Immunohistochemistry for insect Histology and Pathology Diagnosis

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Abstract: Immunohistochemistry (IHC) helps identify different types of tissues and provides important information about the pattern, shape, and structure of cells in a tissue sample. It is a very useful tool and widely used to uncover histology and pathology diagnosis uses. The IHC is a very useful tool to conduct research in the developmental biology area. Some insects, like butterflies, bees, beetles, ants, wasps, and moths, undergo a unique life cycle called complete metamorphosis, which has four distinct stages: egg, larva, pupa, and adult. Larvae are often wingless and have different habits and forms than adults, which are better suited for growth and development. During the metamorphosis, how does the insect legs change is a really an interesting issue. To better understand insect development (1), agriculture pest control (2-5), and insect pathogen interaction (6), here we use an insect Bombyx mori (domestic silk moth) as a model to detail how to conduct the IHC assays, such as HE staining (7), TUNEL to see the cell apoptosis, pH3 and BrdU staining to check cell division (6). The reagents can be easily found, and this step-by-step protocol is easily reproduced.

Keywords: insect morphology; IHC; TUNEL; HE

Procedures

Reagent preparation

PBS (0.01M): 8g NaCl, 0.2g KCl, 1.44g Na_2HPO_4, 0.24g KH_2PO_4, dissolved in 900 ml water, then adjust pH to 7.4 using HCl, finally add water to 1L.

sodium citrate buffer (10mM sodium citrate, 0.025% Triton X-100).

Xylene, 100% ethanol.

Samples preparation step by step.

1. Tissue dissociation.
2. Tissue sample fixation:
3. add fixative (ethanol: chloroform: glacial acetic acid = 6:3:1), change new fixative every 2 or 3 hours, for larger tissues, please change more times, then fix the samples overnight.
4. Dehydration:
5. rinse with 70% ethanol 2 times with 30min each, then 96% ethanol 2 times with 30min each, at last 100% ethanol 2 times with 30min each.
6. Transparency:
7. samples were transparent with xylene 2 times with 30min each.
8. Embedding:
9. First the paraffin was heated at 65 °C, then the samples were embedded in paraffin overnight. Put them on bench to cool down, store in 4 °C for long term use.
10. slicing, spreading and preservation:

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11. Trim paraffin blocks as needed; next the blocks were cut into 5 μm thickness with a microtome machine. All slices are put on the glass slides with gelatin, then put them on slide warmer for 15min to stretch the paraffin samples, then cool down at room temperature, wipe the gelatin, and warm the slides for 24h.
12. deparaffinization:
13. samples were rinsed with xylene 2 times with 10min each.
14. Rinse with 95% ethanol 2 times with 5min each.
15. Rinse with 80% ethanol 2 times with 5min each.
16. Rinse with 70% ethanol 2 times with 5min each.
17. Rinse with water 2 times with 3min each.
18. Rinse with PBS one time.

HE staining

1. Stain with hematoxylin for 5 to 10min.
2. Discard the hematoxylin and rinse with water for 10min.
3. Rinse with 95% ethanol for 5s.
4. Stain with eosin for 30s to 2min.
5. Rinse with 70% ethanol 2 times for 3min each.
6. Rinse with PBS 2 times.
7. Stain with DAPI for 10min.
8. Discard the extra DAPI with PBS.
9. Put on the coverslip.
10. Microscopy detection.

Immunohistochemical staining

11. Low pH sodium citrate buffer for 15min.
12. PBS wash 2 times, 1min each.
13. First antibody for 1h.
14. PBS wash 3 times, 1min each.
15. Fluorescence labelled second antibody for 1h.
16. PBS wash 3 times, 1min each
17. Stain with DAPI for 10min.
18. Discard the extra DAPI with PBS.
19. Put on the coverslip.
20. Microscopy detection.

For the TUNEL staining and BrdU staining, they are the same way as above.

Results

1. Lavel morphology

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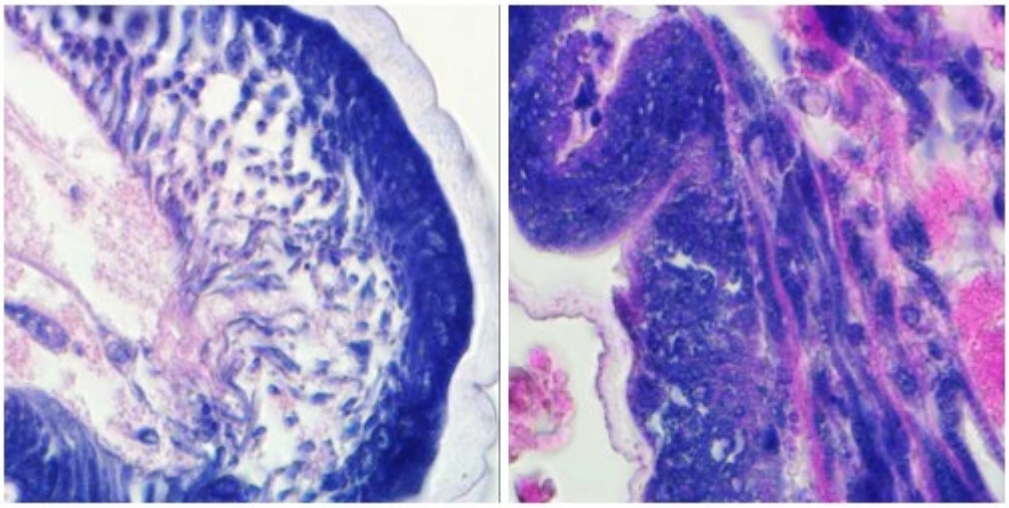
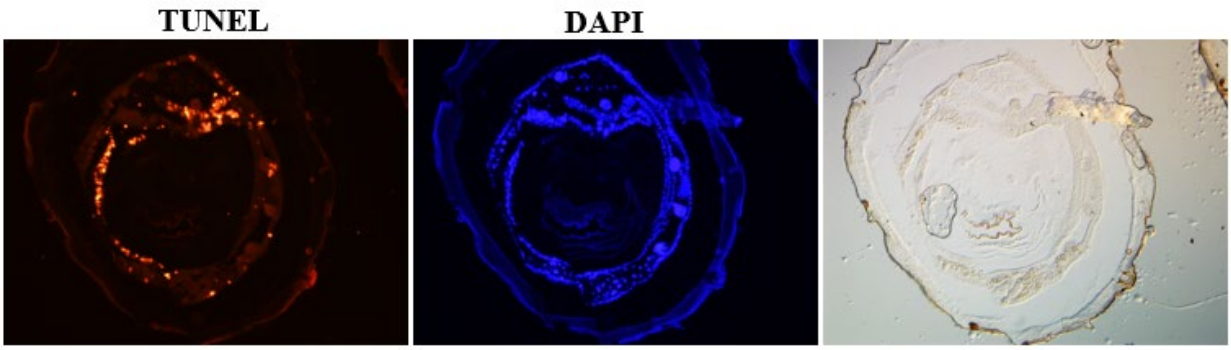
2. HE staining



3. TUNEL staining

TUNEL staining to check the apoptosis during leg phase change.



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