Aquaculture and Fisheries xxx (2017) 1–5

Contents lists available at ScienceDirect

Aquaculture and Fisheries

journal homepage: www.keaipublishing.com/en/journals/aquaculture-and-fisheries/

Short communication

Application of immunohistochemistry technique in hydrobiological studies

Hui Zhang [a, b, 1], Wen Wen [a, b, 1], Jizhou Yan [a, b, *]

[a] Key Laboratory of Exploration and Utilization of Aquatic Genetic Resources, Ministry of Education, Shanghai Ocean University, Shanghai 201306, China
[b] Institute for Marine Biosystem and Neuroscience, Department of Biology, College of Fisheries and Life Sciences, Shanghai Ocean University, Shanghai 201306, China

ARTICLE INFO

Article history:
Received 6 April 2016
Received in revised form
31 August 2016
Accepted 8 September 2016
Available online xxx

Keywords:
Immunohistochemistry
Protocol
Transgenic fish
Hydrobiology

ABSTRACT

There is growing demand for biotechniques in oceanobiological and hydrobiological studies. Immunohistochemistry (IHC) combines anatomical, immunological and biochemical techniques to identify specific antigen-antibody interactions. Detecting the target antigen with labeled antibodies is a multi-step process that requires optimization at every level to maximize the signal detection. Here we use zebrafish samples and optimize each IHC step, particularly antigen retrieval, transgenic reporter double staining and background counterstaining. The resulting images with high signal intensity lead us to recommend the resulting protocols for experiments with fish.

1. Introduction

A large proportion of all life on earth lives in the ocean and fresh water. Aquatic life is a vast resource, providing food, medicine, and raw materials. Many species are important model organisms and are used to understand particular biological phenomena and discoveries. It is becoming increasing clear that metabolic and developmental pathways are conserved over the course of evolution. Therefore, studies on model organisms will provide insight into the workings of other organisms. Recent high throughout biotechnology has developed a huge amount of data and its interpretation and analyses, particularly in relation to potential target biomolecules still remains effectively unexplored.

Immunohistochemistry (IHC) is a technique used to identify the location and distribution of target antigens in cells or tissues by staining with specific antibodies. The antibody is conjugated to either a fluorescent or enzymatic label. Under a microscope the location of the label approximates to the position of the target antigen. Compared to other molecular and cellular techniques, IHC is able to visualize the distribution and localization of differentially expressed proteins within cells and in the proper tissue context.

Nowadays IHC is a routine and essential tool in diagnostic and research laboratories, and is used in a wide range of applications from human to aquatic life studies (Al-Hussinee et al., 2011; Duraiyan et al., 2012). It is our intention to apply IHC techniques in aquaculture and fishery investigations.

2. Principles of immunohistochemistry

Immunohistochemistry is carried out by exploiting the principle that antibodies bind specifically to an antigen in biological tissues, for example in the jaw, gonads or heart. The antigen-antibody interactions can be visualized by a marker including fluorescent dyes, enzyme-substrate color reactions, radioactive elements or colloidal gold. With progress in biotechnology, researchers have been making significant improvements in protein conjugation, tissue fixation methods, detection labels and microscopy. However, IHC tests may give rise to false positive and false negative results due to a multitude of different factors including, fixation, cross-reactivity, antigen retrieval, sensitivity of antibodies, etc. Multiple approaches and permutations have been reported in IHC methodology. Users can optimize IHC procedures to their experimental requirements by targeting, a) fixation by modifying the fixation methods used; b) antigen retrieval to unmask epitopes lost during chemical cross-linking during fixation; c) tissue embedding using

* Corresponding author. 999 Huchenghuan Road, Shanghai Ocean University, Shanghai 201306, China.
E-mail address: jyan2@shou.edu.cn (J. Yan).
[1] These two authors contributed equally to this work.

http://dx.doi.org/10.1016/j.aaf.2017.04.004

alternative approaches best suited to the antigen under study; d) sectioning and mounting, which affects resolution and detail during microscopy and image acquisition; e) quenching/blocking endogenous target activity to prevent false positives and reduce high background using blocking buffers; f) the visualization method used enzyme, fluorophore, colloidal gold or biotin will depend on the target antigen and level of detail required (microscopic or ultrastructure; g) counterstaining to give contrast to the primary stain. Many of these stains are cell structure-specific. A vast array of chromogenic and fluorescent dyes is available to fit every experimental design. Last but not least, antisera must be validated before use in IHC. A variety of methods have been reported for antibody validation, but there are no universally accepted guidelines or standardized methods for determining the validity of these reagents (Bordeaux et al. 2010). By any means, it must be shown that the antibody is specific, selective, and reproducible in IHC. The key to proving antibody specificity is often the correct use of negative controls, including no primary antibody or a cell line or tissue that is known not to express the target protein.

3. Protocol

3.1. Materials and reagents

3.1.1. Zebrafish and cell line

189 transgenic zebrafish was introduced from Dr. Bo Zhang Laboratory (Shao & Yan, 2014). The zebrafish cell line was kindly gifted from Dr. Shawn Burgess (Yan et al., 2006).

3.1.2. Glass slides and culture plate

Adhesion microscope slides (188,105, Citoglas, Haimen, Jiangsu, China); Labtek II chamber slide w/cover (154,526, Thermo Fisher Scientific, Rochester, NY, USA); Coverslip (Circles No. 1–0.13–0.17 mm thick; Size: 22 mm, 12-545-101, Thermo Fisher Scientific); Multi-well plate with 6 wells (140,675, Thermo Scientific, Suzhou, China).

3.1.3. Fixative

Paraformaldehyde (PFA) 32% solution, EM grade (15,714, Electron Microscopy Science, Hatfield, PA, USA). Alternatively, 4% Paraformaldehyde Fix Solution (E672002-0100) available from Sangon Biotech, Shanghai, China.

3.1.4. Blocking and permeabilization buffer

Tissue blocking and permeabilization buffer: 0.3% TritonX-100 (*v*/*v*) and 1% bovine serum albumin (BSA) (*w*/*v*) in 1 × PBS (pH 7.4), 0.01 M; Cell permeabilization buffer: 0.5% TritonX-100 (*v*/*v*) in 1 × PBS (pH 7.4); Cell blocking buffer: 1%BSA (*w*/*v*) in 1 × PBS.

3.1.5. Antibody buffer

10% serum from host species of secondary antibody in PBS, or 2% BSA in PBS.

3.1.6. Antigen retrieval buffer

1 × IHC citrate antigen retrieval buffer (pH 6.0) (cw0128, CWBiotech, Beijing, China).

3.1.7. Primary antibodies

Anti-alpha smooth muscle actin antibody (ab97375, Abcam, New Territories, HK); goat-anti-Sox9b (Zebrafish) (ER14-1692, RayBiotech); mouse-anti-Foxo1b-epitope1 (Abmart, Shanghai, China); mouse-anti-Foxo1b-epitope6 (Abmart, Shanghai, China).

3.1.8. Secondary antibodies

Chicken anti-rabbit IgG (H + L) secondary antibody, Alexa Fluor 488 conjugate (A21441, ThermoFisher); goat anti-mouse IgG, DyLight488 conjugate (CW0150, CWBiotech); donkey anti-Goat IgG, Rhodred-X conjugate (CW0212, CWBiotech, Beijing, China).

3.1.9. Mounting solution

50% glycerol in 0.5 M carbonate buffer (pH 9.0–9.5); Fluoroshield™ with DAPI (F6057, Sigma-Aldrich, St. Louis, MO, USA); Hoechst stain solution (H6024, Sigma-Aldrich, St. Louis, MO, USA). Alternatively DAPI Stain Solution (E607303-0002) and Histochoice® mounting media (A600157-0100) available from Sangon Biotech, Shanghai, China (see Fig. 1).

3.2. Procedures

3.2.1. IHC for paraffin sections (Fig. 1) (Zhang et al., 2015)

1) Fix tissue with fresh 4% PFA in PBS (pH 7.4, 4 °C overnight. 1 × PBS rinse five times, 5 min each.
2) Dehydrate with alcohol 70% (6 min), 80% (6 min), 95% (5 min), 95% (5 min), 100% (1 min), 100% (1 min).
3) Clear in 100% alcohol: xylene 1:1 mix (5 min), xylene I (1 min), xylene II (2 min).
4) Infiltrate with paraffin wax (56 °C) twice, 1 h each time.
5) Embed tissue in paraffin blocks. Store at 4 °C until sectioning.
6) Prepare sections (5 μm) of the tissue embedded in paraffin and float the sections on water at 38 °C.
7) Use an adhesive glass slides (suitable for IHC) to collect the floating paraffin section and then position the section using a brush.
8) Dry sections at 37 °C overnight.
9) Dewax the sections in 2 changes of xylene, 5 min each.
10) Rehydrate with alcohol 100% (5 min), 95% (5 min), 80% (3 min), 70% (3 min) and finally place in distilled water (2 min).
11) Antigen retrieval: Immerse in 1 × IHC citrate antigen retrieval buffer (pH 6.0) and place in an autoclave at 121 °C for 3 min. Allow the buffer and slides to cool down to room temperature (RT).
12) Wash three times in PBS (pH 7.4), 5 min each.

Label the slides with a solvent resistant pen and demarcate the tissue if required. Add blocking buffer (1%BSA + 0.3%TritonX-100, PBS) to the tissue section and incubate at RT for 1 h.

13) Remove the excess blocking buffer and then incubate the sections with primary antibody in a humid box at 4 °C for 12–16 h.
14) Remove excess antisera and then wash in PBS three times, 10 min for each wash. Add 2nd Fluorescence-conjugated antibody in a dark room and incubate at 25 °C for 1 h.
15) Remove excess the 2 nd antibody and wash the slide in PBS three times, 10 min for each wash. Counterstain the section and/or add a mount solution with counterstaining dye (see Notes)

3.2.2. IHC for cryosections of transgenic fish tissues expressing a reporter (GFP) gene (Fig. 2)

This IHC technique has been verified in Flk1-GFP transgenic zebrafish, by which Flk1-GFP signal and anti-GFP antibody staining are well matched (Zhang et al., 2015) (see Table 1).

To avoid quenching of fluorescence (GFP) by ethanol and acetone, the use of frozen sections of GFP-reporter transgenic fish is recommended:

Please cite this article in press as: Zhang, H., et al., Application of immunohistochemistry technique in hydrobiological studies, Aquaculture and Fisheries (2017), http://dx.doi.org/10.1016/j.aaf.2017.04.004