Jan 08, 2026

Immunohistochemistry and immunofluorescence protocols

DOI
https://dx.doi.org/10.17504/protocols.io.ewov1ko5kgr2/v1

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OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.ewov1ko5kgr2/v1

Protocol Citation: Junyu Xiang 2026. Immunohistochemistry and immunofluorescence protocols. **protocols.io** https://dx.doi.org/10.17504/protocols.io.ewov1ko5kgr2/v1

Protocol status: Working
We use this protocol and it's working

Created: January 08, 2026

Last Modified: January 08, 2026

Protocol Integer ID: 238193

Keywords: immunofluorescence protocol, immunohistochemistry, immunofluorescence, phosphorylation level, indicated protein, phosphorylation, ihc, such as nprl2

Abstract

A detailed description for immunohistochemistry (IHC) and immunofluorescence (IF) experiments. The expected results can clearly demonstrate the indicated proteins and their phosphorylation levels, such as NPRL2 and pS235/236-S6.

Immunohistochemistry protocol

1 Formalin-fixed paraffin-embedded specimens or organoids are cut into 5-10 µm thick sections.

2 The sections are deparaffinized, rehydrated, and blocked with 3% H_2O_2 for 15 min.

3 To remove melanin in immunohistochemistry of melanoma tumor tissues, sections are emersed in 0.5% permanganate potassium solution at 55°C for 3 minutes, then treated with 1% oxalic acid solution for 3 cycles of 30 seconds followed by water rinse for 1 minute.

4 Tissue antigens are retrieved by heating the sections in a pressure cooker for 150 sec.

5 After cooling to room temperature, each section is incubated with primary antibody at 4°C overnight, and then with goat secondary antibody against rabbit and mouse immunoglobulins (DAKO).

6 For visualization, 1-5% 3,3'-diaminobenzidine tetrahydrochloride is used as a chromogen.

7 After washing with water, the sections are counterstained with hematoxylin.

8 Negative control slides with the primary antibody replaced with phosphate-buffered saline (PBS) are included.

9 All immunostained sections are independently evaluated by two pathologists blinded to the patients' clinical status, with interobserver discrepancy being less than 10%. In the case of discrepancy, a consensus interpretation is reached with a third pathologist.

10 The quantification method is based on cell number counting, or multiplicative index of the average staining intensity (score 0-3) and extent of staining (score 0, 0%; 1, 0-25%; 2, 25-50%; 3, 50-75%; 4, 75-100%) in the cores (such as pS6 IHC score), yielding a staining index ranging from 0 (no staining) to 12 (extremely extensive, extremely strong staining).

Immunofluorescence protocol

11 Tissue sections are permeabilized with PBS-T (containing 0.25% Triton X-100) for 30 minutes and blocked with 5% goat serum in PBS for 60 minutes.

12 Primary antibodies are diluted in TBS-T (containing 0.1% Tween-20) (dilution 1: 25~200) and incubated at 4°C overnight, followed by incubation with appropriate secondary antibodies

13 Coverslips are mounted on slides using anti-fade mounting medium with DAPI.

14 Images are acquired with a ZEISS 880 Microscope.

15 For multiple-immunofluorescence for samples array, Opal 7-color Manual IHC Kit (NEL801001KT, PerkinElmer) is used and performed according to manufactures instructions.

16 **Fluorescence signal**s were detected by Vectra Polaris Automated Quantitative Pathology Imaging System
(PerkinElmer) and the data was analyzed by StrataQuest (ver. 7.0.1.176, TissueGnostics).

17 Pearson's correlation in cells were quantified by the Coloc2 plugins in ImageJ (ver. 1.53q).