

Materials and methods

IHC protocol. Fixative used, including concentration, temperature and duration, resin, thickness of sections OR freezing temperature and thickness of sections

Fixing used: 10% neutral buffered formalin

Concentration: 10% (v/v)

Temperature: room temperature (25°C)

Duration: 12 h

resin: paraffin

Slice thickness: 4 μm

- For paraffin-embedded samples: antigen retrieval-heating temperature, washing reagent and rehydration in descending alcohol series

Paraffin embedding:

Bake the slices in a 60°C oven for 60 min.

Soak in xylene for 3 times for 10 min each time.

Hydration in decreasing gradient alcohol: 100% ethanol twice \rightarrow 95% ethanol \rightarrow 70% ethanol for 5 min each time.

Rinse under running tap water for 10 min.

Rinse with 1X PBS buffer (pH 7.4) for 3 times, each time for 5 min.

Antigen repair

Methods: Heat-induced epitope repair

Repair buffer: sodium citrate repair solution (10 mM sodium citrate, pH 6.0) or EDTA repair solution (1 mM EDTA, pH 8.0).

Heating equipment: pressure cooker or microwave oven

Heating temperature and time: after boiling in the pressure cooker, heat for 2 min, or in the microwave oven, heat to boiling with high fire, then turn to medium-low fire and keep boiling for 15 min.

Cooling: naturally cool to room temperature (about 30-60 min).

Washing: Rinse with 1X PBS buffer for 3 times, 5 min each time.

- For intracellular antigens or membrane proteins with an internal epitope: Permeabilization reagent and concentration

Permeation reagent: Triton X-100

Concentration: 0.1% (v/v) in 1X PBS.

- Blocking reagent and percentage, temperature and duration

Blocking reagent: normal goat serum.

Concentration/percentage: 5% (v/v) in 1X PBS.

Temperature and duration: Incubate at room temperature for 60 min.

- If HRP/DAB was used: Quenching step-name and concentration of reagent used to block endogenous peroxidase/phosphatase activity

Quenching reagent: 3% hydrogen peroxide (H_2O_2) solution. Preparation: dilute 30% H_2O_2 stock solution with methanol or ultrapure water.

- Primary antibody dilution, catalogue number, supplier, and temperature and duration of incubation
- Secondary antibody dilution, catalogue number, supplier, conjugate [e.g. Fluorophore, biotin/streptavidin or enzyme (peroxidase/phosphatase)], and temperature and duration of incubation

Primary antibody: name of target protein

Dilution: 1:200 (in 1X PBS or antibody diluent)

Catalog number: ab92742

Supplier: Abcam

Incubation conditions: temperature: 4°C.

Duration: overnight (about 16 h)

Secondary antibody: Goat Anti-Rabbit IgG HRP

Dilution: 1:500 (in 1X PBS or antibody diluent)

Catalog number: ab6721

Supplier: Abcam

Conjugate: peroxidase

Incubation conditions: room temperature duration: 60 min.

- If HRP/DAB was used: Chromogen detection - name of the detection reagent Counterstain

Detection reagent: DAB (3,3'-diaminobenzidine) substrate chromogenic kit.

Name/supplier: dab substrate kit, cat. # sk-4100, vector laboratories.

Double dyeing: hematoxylin

- Type of microscope (light, fluorescence or confocal) and magnification or scale bar in figure/legend
- Software used for analysis (name, version and manufacturer)

Microscope type: upright optical microscope

Imaging parameters: 20x

Scale: 50 μm

Name of analysis software: ImageJ (Fiji)

Version number: ImageJ Fiji (based on ImageJ 1.53t)

Manufacturer/:[<https://imagej.net/software/fiji/>] (<https://imagej.net/software/fiji/>)

Genetic analysis protocol.

- Name of the company if applicable Shanghai YiJian inspection co., ltd
- The name, catalogue number and the supplier of the kit used to prepare DNA/RNA samples for sequencing.

Name: QIAamp DNA Blood Maxi Kit

Catalog number: 51194

Supplier: QIAGEN

- The method used to verify quality/integrity of the processed samples.

Method 1: Measurement of concentration and purity

Instrument/Kit: NanoDrop One/OneC Micro Ultraviolet Spectrophotometer.

Supplier: Thermo Fisher Scientific

Evaluation standard: use 1.5-2.0 μl samples to measure the concentration ($\text{ng}/\mu\text{l}$) and purity. Qualified DNA samples should meet the following requirements: the ratio of A260/A280 is between 1.8 and 2.0, and the ratio of A260/A230 is greater than 2.0.

Method 2: Integrity and quantitative confirmation

Instrument/kit: Qubit 4 fluorometer and Qubit dsDNA HS Assay Kit.

Catalog number: Q33230 (instrument), Q32851 (kit)

Supplier: Thermo Fisher Scientific

Evaluation criteria: Accurate DNA concentration ($\text{ng}/\mu\text{l}$) was obtained quantitatively by fluorescence method. At the same time, it is suggested to use agarose gel electrophoresis or Agilent bioanalyzer to confirm the integrity of DNA, and the main band should be greater than 10 kb, with no signs of degradation.

- The type of sequencing, including nucleotide length and the direction of sequencing.

Type: whole exon group capture and sequencing.

Reading length: Paired-end 150 bp (PE150)

Sequencing direction: two-way sequencing.

- The name, catalogue number and supplier of sequencing kit. This is not the same as the kit used to prepare the sequencing library.

Sequencing kit:

Name: Illumina nova seq 6000s4 Regent Kit (300 cycles)

Catalog number: 20028317

Supplier: Illumina

- The loading concentration of the final library, including how concentrations were measured (M not g)

Measurement method: Real-time fluorescence quantitative PCR, which is the most accurate method for sequencing and quantification at present, because it only detects amplified library fragments with complete adapters.

Typical loading concentration: It depends on the Illumina sequencer and the type of flow cell used. For NovaSeq

6000 S4 flow cell, the final library loading concentration is 200-300 pM. (1 nM=1,000 pM)

- Software used to analyse data (name, version number, URL/reference/supplier).

A. Quality control and comparison of raw data

Software name: FastQC

Version number: 0.11.9

URL:<https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>

B. sequence alignment and pretreatment

Software name: BWA-MEM (Burrows-Wheeler Aligner)

VersionNo.: 0.7.17

URL/ source: [<http://bio-BWA.SourceForge.net/>] (<http://bio-BWA.SourceForge.net/>).

C. Annotation and interpretation of variation

Software name: ANNOVAR

VersionNo.: (2020-06-08)

URL:<https://annovar.openbioinformatics.org/en/latest/>