

# Immunohistological Diagnosis



# ANTIBODY TYPES

- IgA
- **IgE**
- **IgD**
- **IgG**
- **IgD**
- **IgM**



# STRUCTURE OF ANTIBODY

- Antibodies are heavy (~150 kDa) globular plasma proteins. The basic structure of all antibodies are same.
- There are four polypeptide chains: two identical heavy chains and two identical light chains connected by disulfide bonds.  
Light Chain (L) consists polypeptides of about 22,000 Da and Heavy Chain (H) consists larger polypeptides of around 50,000 Da or more. There are five types of Ig heavy chain (in mammal) denoted by the Greek letters:  $\alpha$ ,  $\delta$ ,  $\epsilon$ ,  $\gamma$ , and  $\mu$ . There are two types of Ig light chain (in mammal), which are called lambda ( $\lambda$ ) and kappa ( $\kappa$ ).

# STRUCTURE OF ANTIBODY

- An antibody is made up of a variable region and a constant region, and the region that changes to various structures depending on differences in antigens is called the variable region, and the region that has a constant structure is called the constant region.



# IHC – THE BASIC STEPS

A general IHC protocol consists of four main steps:

- (1) fixation – to keep everything in its place,
- (2) antigen retrieval – to increase availability of proteins for detection,
- (3) blocking – to minimize pesky background signals and
- (4) antibody labeling and visualization – getting the pretty pictures.



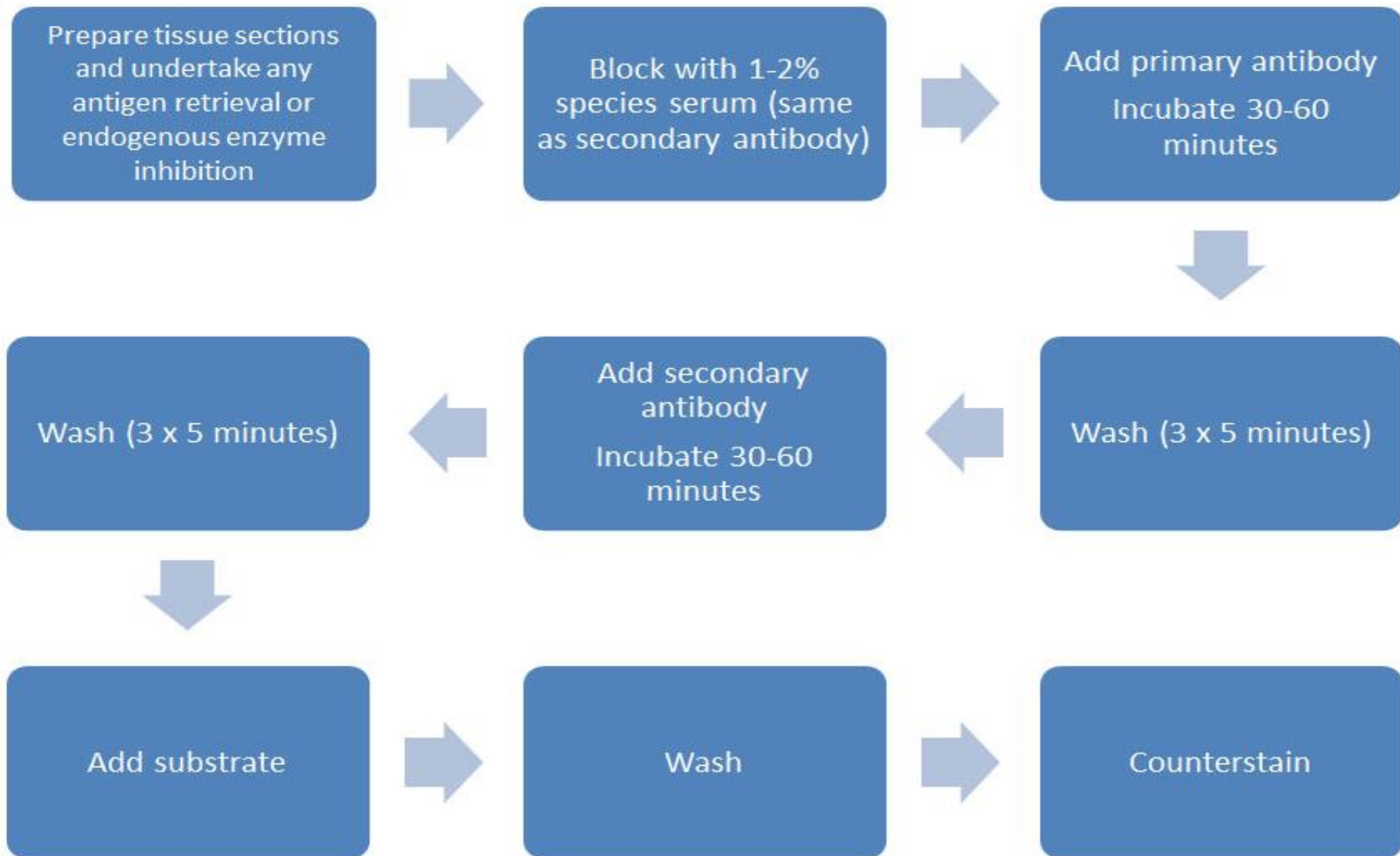
- There are 2 IHC staining methods.

*1- Indirect staining*

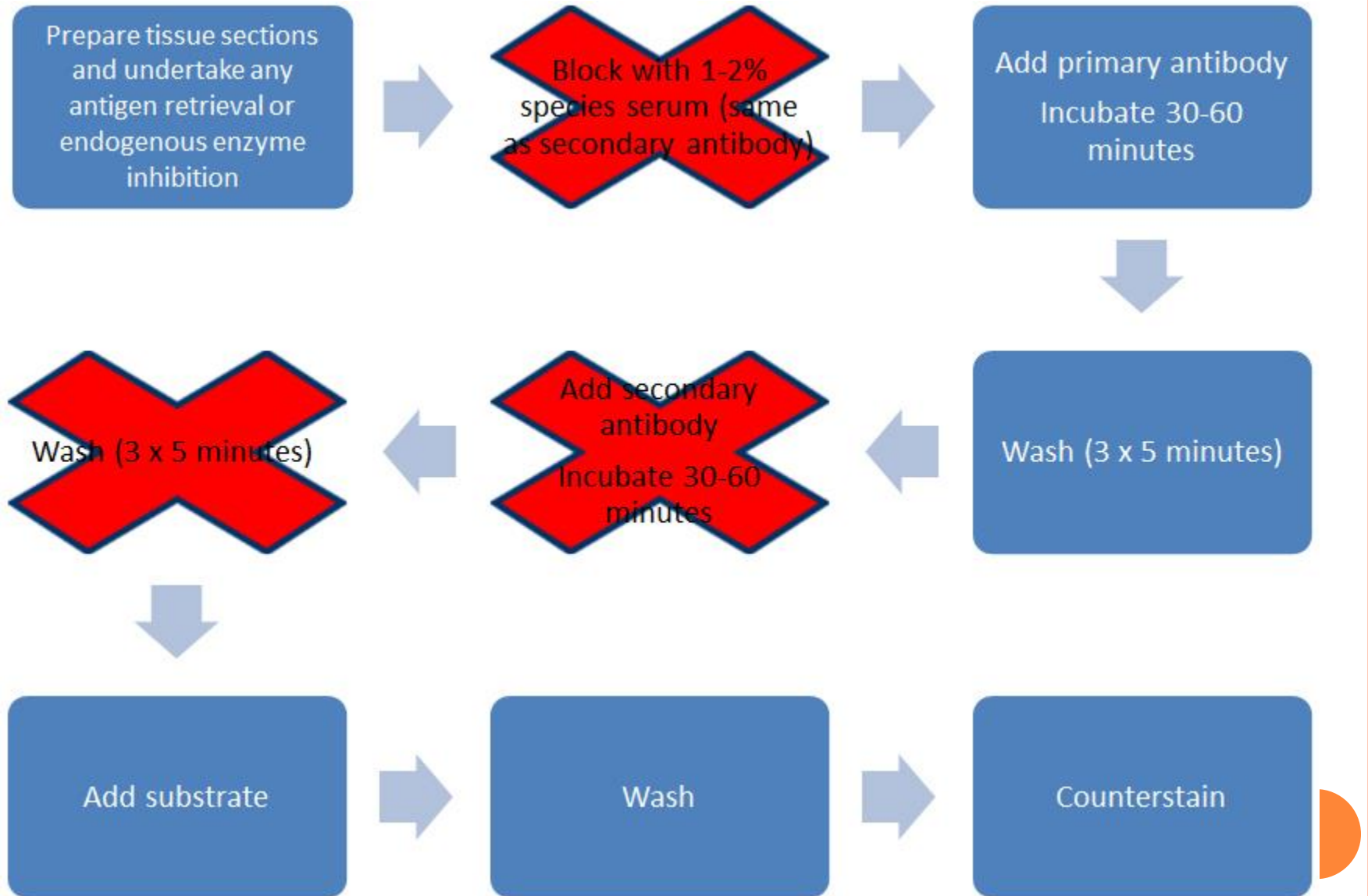
*2- Direct staining*



# Indirect staining



# Direct staining





# Immunohistochemistry Staining Methods

- 1-Avidin-Biotin Immunohistochemistry
- 2-Polymer-Based Immunohistochemistry
- 3-Catalysed Signal Amplification (CSA)
- 4- Fluorescyl-tyramide Amplification
- 5- Improved Catalysed Signal Amplification (iCSA)
- 6- Multi-Staining Immunohistochemistry
- 7- Immunofluorescence

