

## FAQ - How should I choose my secondary antibodies?

### Classic IgG Based Secondary Reagents

Most ICC and IHC staining protocols rely on secondary antibodies. However, secondary antibodies can cross-react with endogenous immunoglobulins in the tissue or generate false-positive signals through nonspecific binding. In multiplex experiments, species-specific secondary antibodies may also cross-react with primary antibodies raised in other species. To minimize these issues, use secondary antibodies that are pre-adsorbed not only against immunoglobulins of the target species but also against IgGs from the host species of all other primary antibodies used in the experiment.

SYSY Antibodies recommends including a secondary-only control for each sample by omitting the primary antibody in the staining protocol. This control helps identify and prevent false-positive staining arising from the secondary detection system.

### Single Domain Secondary Reagents (FluoTags®)

SYSY Antibodies offers a panel of single-domain secondary reagents that provide outstanding species specificity. Because they are recombinantly produced with a defined amino-acid sequence, these reagents deliver exceptional lot-to-lot consistency.

NanoTag's FluoTag® species-specific anti-immunoglobulin reagents are alpaca single-domain antibodies, also known as Nanobodies® (trademark of Ablynx, Inc.). At approximately 15 kDa, they are about ten times smaller than conventional IgGs. Their monovalent nature allows them to be directly incubated with primary antibodies without forming non-functional aggregates. In addition to reducing hands-on time in immunofluorescence workflows, FluoTags® enable high-precision applications that are difficult or impossible to achieve with conventional secondary antibodies.