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

## **Classic IgG Based Secondary Reagents**

Most ICC and IHC staining methods are based on the use of secondary antibodies. However, secondary antibodies may cross-react with endogenous immunoglobulins in the tissue or produce false-positive staining through non-specific binding of the secondary antibody. In Multiplex experiments, species specific secondary antibodies may cross-react with primary antibodies originating from a different species. Use secondary antibodies that have not only been pre-adsorbed against IgGs of the species to be stained, but also to IgGs of the host species of the other primary antibodies.

Synaptic Systems recommends including a secondary system control for each sample to be stained by omitting the primary antibody in the staining protocol. This controls against false-positive staining from the secondary system.

## **Nanobody Based Secondary Reagents**

SYSY Antibodies offers a panel of <u>nanobody based secondary reagents</u> that provide excellent species specificity. Since they are recombinantly made with a defined sequence, they show unsurpassed lot to lot consistency.

NanoTag's FluoTag® species-specific anti-immunoglobulin secondary tools are alpaca single-domain antibodies, also referred to as Nanobodies® (trademark of Ablynx, Inc). With only around 15 kD, they are 10-times smaller than conventional IgGs. Since Nanobodies are monovalent, they can be directly incubated with primary antibodies without forming non-functional clusters. Besides saving time on immunofluorescence stainings, the FluoTags® enable researchers to perform innovative applications with a precision and quality unreachable using conventional secondary antibodies.

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