



: Mouse
 : Rabbit
 : Goat
 : Rat
 : Suggested starting incubation time

: Antibody
 : Optional Amplification Steps
 : Multimer (Omni/Ultra) against "Species"-HRP

FAQs, Tips, and Tricks

Q. Can I multiplex with same species primary antibodies in sequential steps?

A. Yes, our next-generation chromogens are deposited using TSA deposition so the chromogen signal is covalently bound. This allows mild denaturing conditions to wash off the first primary without affecting signal, and a second primary of the same species can be used in the following step.

Q. Do I have to use fluorescence to visualize co-expression?

A. No, our translucent chromogens allow for co-expression and co-localization without the need for a fluorescent microscope. Further, brightfield also shows hematoxylin counterstain so that the entire tissue context can be seen - something not possible with fluorescence.

Q. What is the best order for chromogens when multiplexing?

A. The rule of thumb is DAB > purple > red > yellow > teal > green > silver. However, localization of each target, sensitivity of each biomarker epitope, and chromogen translucence must also be considered.

Q. What reagents are available for low expressing targets?

A. DISCOVERY HQ products use unique haptens for robust sensitivity. In addition to HQ detection, DISCOVERY amplification kits use both unique haptens and tyramide for enzymatic amplification. The amplification kits can boost signal while maintaining specificity.

Q. What chromogens are best for a co-localization experiment?

A. DISCOVERY Purple, DISCOVERY Yellow, and DISCOVERY Teal are the optimal chromogens for detecting co-localization. DISCOVERY Purple and DISCOVERY Yellow will result in a reddish/orange hue. DISCOVERY Purple and DISCOVERY Teal will result in an indigo blue hue. DISCOVERY Yellow and DISCOVERY Teal will result in a green hue.

Q. Can reagents be added manually (e.g. with a pipette) during an automated IHC run?

A. Yes. Ready-to-use reagents can be registered and used immediately. Third party reagents can be either applied manually or fully automated in a user fillable dispenser. Protocols with manual applications will beep when the reagents need to be applied, allowing the user to manually access the slide to manually pipette on reagents.

Q. How many chromogenic colors can be combined on one IHC?

A. The amount of chromogenic colors that can be combined depends on many variables, including the stability of epitopes being detected, and the compatibility of chromogens being used. Conditions for each target should be optimized individually, then combined and evaluated for equivalent performance.

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