Secondary Antibody Selection for

SPATIAL BIOLOGY

Consider Secondary Host And Target Species:

- Ensure the primary antibody targets the tissue species for accurate detection and the host of the primary antibody is different than the tissue species.
- Choose a secondary antibody from a different species than the tissue to avoid background interference.
- For multiplex imaging, use secondary antibodies that target the primary antibodies from various species, each tagged with distinct fluorophores for multiple antigen detection.

Match Secondary To Primary Class or Subclass:

- For primary polyclonal IgG antibodies, select anti-IgG secondary antibodies.
- For monoclonal antibodies with a specific IgG subtype (i.e, IgG1), use a secondary antibody specific to that subtype (i.e, anti- IgG1).
- Use anti-IgM secondaries for primary monoclonal IgM antibodies.

Select Affinity-Purified Antibodies:

- Affinity purification provides high specificity and low background.
- Ensures consistent quality and reduced nonspecific binding, ideal for low abundance protein detection.

Select The Correct Conjugation:

- Utilize secondary antibodies conjugated to specific fluorochromes like ATTO, DyLight™, or Cy™ for their exceptional brightness and stability.
- Ideal for spatial biology, these fluorochromes enhance visualization of low-expression targets.

Use Cross-Adsorbed Antibodies:

- Cross-adsorption refines antibody specificity by removing cross-reactive components.
- It lowers cross-reactivity to other species, ensuring accurate multi-label experiment results.
- Using cross-adsorbed antibodies minimizes false positives in complex spatial biology studies.

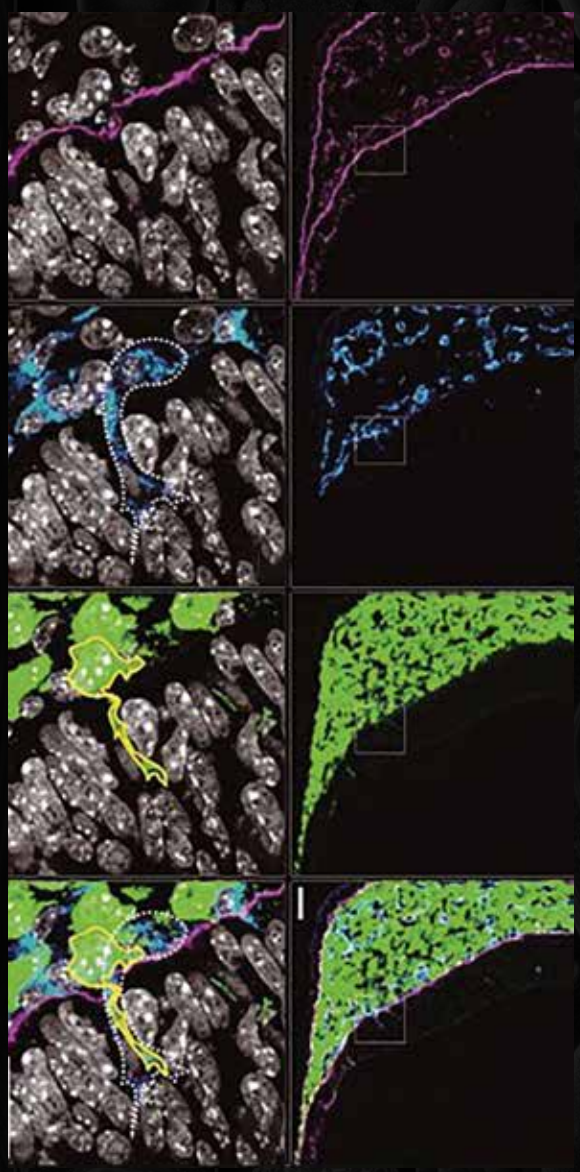

Fig. See neural crest-derived cells infiltrating the brain region in a P0-Cre/EGFP mouse embryo, using specialized staining to track their migration and interaction with endothelial cells through the brain's membrane. Antibody used - Rat IgG (H&L) Antibody DyLight™ 649 Conjugated Pre-Adsorbed Goat Polyclonal.

Antibodies for IF/Multiplex	Host	Part No.
Mouse IgG (H&L) DyLight™ 405 Conjugated Pre-Adsorbed	Goat Polyclonal	610-146-121
Mouse IgG (H&L) DyLight™ 488 Conjugated Pre-Adsorbed	Goat Polyclonal	610-141-121
Human IgG (H&L) DyLight™ 488 Conjugated Pre-Adsorbed	Goat Polyclonal	609-141-123
Rabbit IgG (H&L) DyLight™ 549 Conjugated Pre-Adsorbed	Goat Polyclonal	611-142-122
Chicken IgG (H&L) DyLight™ 549 Conjugated Pre-Adsorbed	Goat Polyclonal	603-142-126
Rat IgG (H&L) DyLight™ 549 Conjugated Pre-Adsorbed	Goat Polyclonal	612-142-120
Golden Syrian & Armenian Hamster IgG (H&L) DyLight™ 649 Conjugated Pre-Adsorbed	Goat Polyclonal	620 -143-440
Guinea Pig IgG (H&L) DyLight™ 649 Conjugated Pre-Adsorbed	Goat Polyclonal	606-143-129
Human IgG (H&L) DyLight™ 680 Conjugated Pre-Adsorbed	Goat Polyclonal	609-144-123
Rabbit IgG (H&L) DyLight™ 800 Conjugated Pre-Adsorbed	Goat Polyclonal	611-145-122

DyLight™ conjugated pre-adsorbed secondary antibodies, including donkey host polyclonals reactive to various species, are available. Visit our website for the complete list of fluorescent conjugated antibodies.

Carrier-free Antibodies	Host	Part No.
Anti-BRCA1	Mouse Monoclonal	ABIN1724848
Anti-BRCA2	Rabbit Polyclonal	ABIN673434
Anti-CA19-9	Rabbit Monoclonal	ABIN6936552
Anti-CA125	Rabbit Polyclonal	ABIN724760
Anti-CEA	Rabbit Polyclonal	ABIN7437728
Anti-CEACAM5	Rabbit Polyclonal	ABIN2856631
Anti-HER2	Mouse Monoclonal	ABIN1383851
Anti-KIT	Rabbit Polyclonal	ABIN1387260
Anti-MAP2	Mouse Monoclonal	ABIN7456158
Anti-Osteocalcin	Rabbit Polyclonal	ABIN1385851

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Rockland is a global biotechnology company manufacturing leading-edge tools for basic, applied, and clinical research in many industries. Tomorrow's discoveries demand the rigor and keen dedication to the quality delivered by Rockland. Established in 1962, Rockland is a privately held company, operating manufacturing facilities with antibody and protein production capabilities near Philadelphia, Pennsylvania, USA.

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10 tips for
LATERAL FLOW ASSAY DEVELOPMENT

Tip 01

1. Knowing your target is the key to success

With LFAs, you can detect a protein, bacterium, virus, other biomolecule, or chemical moiety. The identity of the target analyte determines the overall design of the LFA. Different analytes may require specific assay configurations, including the choice of antibodies or other recognition elements, conjugation methods, and detection strategies.

Tip 02

2. Consider the sample type

Different sample types (such as blood, urine, saliva, tissue and cell extracts, or environmental samples of various sources) may require specific adaptations to sample handling. Complex samples, such as urine or blood, can cause matrix effects or alter viscosity, causing false positive or negative results. Different buffers should be tested to ensure optimal performance in LFAs.

Tip 03

3. Your target and nothing else

Specific antibodies contribute to the accuracy of the assay by preventing cross-reactivity with other substances that might be present in the sample. This is especially important in cases where the concentration of the target is low, and accurate detection is crucial. Testing for selection of antibodies often requires more than ELISA and may include other methods, such as bio-layer interferometry (BLI).

BLI Services

Tip 04

4. Stability matters

The appropriate antibody must have good stability for long-term storage. The specific conditions of the LFA, such as temperature, pH value, and other reagents, must also be considered.

Tip 05

5. Conjugation suitability

Conjugation is also an important point. The detection antibody must be able to easily and consistently conjugate with markers, such as nanoparticles (gold and colored latex or carboxymethylcellulose) or dyes. Keep in mind that the binding affinity must not be compromised in the process.

Tip 06

6. Decide monoclonal or polyclonal

Monoclonal antibodies show high specificity for a particular epitope on the target and low cross-reactivity. To compensate the lower sensitivity, multiple mAbs can be combined in an assay. Polyclonal antibodies are often advantageous because they are less expensive and have higher affinities for the antigen due to their ability to recognize different epitopes. Rigorous testing is the prerequisite for finding a suitable pair for your assay.

See products

Tip 07

7. Select your materials carefully

Hydrophilic properties of the materials used in the LFA are crucial for promoting capillary action and maintaining an appropriate flow rate. The membrane serves as the substrate for immobilizing the capture and detection components. The material and surface properties of the membrane should be chosen to facilitate proper binding, ensuring the stability and functionality of the immobilized reagents.

Tip 08

8. Think about the future

Long-term availability of antibodies ensures economic stability and predictability in the production of LFAs. The use of consistent antibody batches over a long period of time ensures the reproducibility of the test results and stability of quality control during production. For this reason, use of recombinant antibodies is desirable.

See custom services

Tip 09

9. Learn from others

Reviewing scientific publications and reports where the antibody has been used successfully for similar applications may help save time and money for antibody validation.

Tip 10

10. Consider cost and quality

Evaluate the cost of the antibodies and the overall production process to ensure the LFA remains cost effective while meeting performance requirements. The LFA format (e.g., type of cassette or membrane holder) and sample handling accessories (e.g., collection and mixing tubes, buffers, etc.) may also significantly impact pricing. Work closely with reliable antibody suppliers or manufacturers to ensure that the antibody meets the desired requirements.

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