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Product Information

Labeled Rabbit Anti-Goat IgG (H+L) Antibodies

Cat. No.	Label	Unit Size
L112A	Andy Fluor 488	100 µg
L112B	Andy Fluor 488	500 µg

Spectral Properties:

Label	Ex nm	Em nm
Andy Fluor 488	495	520

Storage upon receipt:
- **2-8°C**
- **Protect from light**
- **Avoid freeze-thaw cycles**

Product Description

Fluorescent Rabbit Anti–Goat IgG conjugates are ideal for fluorescence microscopy and confocal laser scanning microscopy, flow cytometry, and fluorescent western detection.

The Rabbit Anti–Goat IgG antibody conjugates are prepared from affinity purified antibodies. To minimize cross-reactivity, the rabbit anti–goat IgG antibodies have been adsorbed against human IgG and human serum prior to conjugation. The degree of labeling for each conjugate is typically 2–8 fluorophore molecules per IgG molecule. At the time of preparation, the products are certified to be free of unconjugated dyes and are tested in an immunofluorescence experiment to ensure low nonspecific staining.

Product Specification

Physical State: Lyophilized power

Buffer: PBS, pH 7.4

Stabilizer: 0.1% BSA

Preservative: 0.02% Sodium Azide

Reconstitution and Storage: Store lyophilized power at 2-8ºC. When ready to use, rehydrate with dH_2O (50 µL for 100 µg antibody or 250 µL for 500 µg antibody) to make 2 mg/mL solution and centrifuge if not clear. Product is stable for about 6 months at 2-8ºC as an undiluted liquid. Prepare working dilution fresh each day. For extended storage after rehydration, add an equal volume of glycerol for a final concentration of 50%, and store at -20 ºC as a liquid.

Guidelines for Use

Centrifuge the reconstituted protein conjugate solution briefly in a microcentrifuge before use. Add only the supernatant to the experiment. This step eliminates any protein aggregates that may have formed during storage, thereby reducing nonspecific background staining.

Because staining protocols vary with application, the appropriate dilution of antibody should be determined empirically. For the fluorophore- and biotin-labeled antibodies, a final concentration of 1–10 µg/mL should be satisfactory for most immunohistochemical applications. For flow cytometry applications, 0.06–1.0 µg per 1×10^6 cells should yield satisfactory results.

General Protocols

Immunofluorescence Protocol for Microscopy

There are many methods for immunofluorescence staining. The protocol below is a general guideline for staining cells and should be optimized or modified to obtain the best results for each particular application.

1. Coverslip preparation for adherent cells

1.1 Culture cells on slide chambers or sterile glass coverslips We recommend 18 x 18 mm square coverslips in 6-well plates or 4-well chamber slides.

1.2 Allow cells to adhere and treat as desired.

1.3 Rinse cells gently with PBS.

2. Coverslip preparation for non-adherent cells

2.1 Coat coverslips with 0.01% poly-L-lysine solution for 10 minutes at room temperature.

2.2 Aspirate the poly-L-lysine solution and allow coverslips to dry completely.

2.3 Centrifuge cells in medium and resuspend in PBS. Transfer cells to coverslips.

2.4 Incubate for 30-60 minutes. Check for adherence by microscope.

3. Fixation and Staining

3.1 Fix with 4% paraformaldehyde/PBS, 15 min.

3.2 Rinse twice with PBS to remove traces of fixative.

3.3 Permeabilize with 0.1 - 0.5% TritonX-100/PBS, 5-10 min.

3.4 Block with blocking agent such as with 5% BSA or normal goat serum in PBS, 30 min.

3.5 Dilute primary antibody in dilution buffer as recommended in the specific product's datasheet. Overlay enough diluted antibody to cover cells on coverslip (150-200 µL is usually sufficient to cover the surface area) or add to each chamber of the chamber slides. Keep slips covered or in a humidified chamber to avoid evaporation.

3.6 Rinse three times with PBS, 5 min each wash.

3.7 Dilute fluorescent secondary antibody in dilution buffer and incubate for 1 hour at room temperature. General range for IgG conjugates is between 1-10 µg/mL for most applications. Cell samples without primary antibody incubation is recommended for background control. Keep slips covered or in a humidified chamber to avoid evaporation.

3.8 Rinse three times with PBS, 5 min each wash.

3.9 Additional staining with fluorescent nuclear stains or phalloidins can be done at this step.

3.10 Invert each coverslip onto a pre-cleaned slide with fluorescence anti-fade mounting media. Seal edges with clear polish if desired.

3.11 Store slides in the dark at 4°C.

Staining Protocol for Flow Cytometry

There are many alternative procedures that can be used for specific staining experiments. The protocol below is a general guideline for flow cytometry and should be optimized or modified for each application.

1. Aliquot 1 X 10^6 cells into 12 X 75 mm polypropylene tubes for flow cytometry.
2. For intracellular staining, cells can be fixed first to ensure stability of soluble antigens or antigens with short half-lives. We recommend a fix and perm kit from reliable manufacturers. Follow manufacturer's instructions.
3. Add the primary antibody or isotype control at the appropriate dilution to the assay tubes. Incubate according to manufacturer's instructions.
4. Rinse cells twice by centrifugation with 2-3 mL incubation buffer.
5. Decant supernatant and re-suspend the pellet in remaining volume of wash.
6. Add fluorescent secondary antibody and incubate for 20-30 minutes. General range for secondary antibodies is between 1-10 µg/mL for IgG conjugates for most applications.
7. Rinse cells twice by centrifugation with 2-3 mL incubation buffer. Centrifuge to collect cells after each wash. Decant supernatant.
8. Resuspend cells in 0.5 mL of diluent of choice to analyze on flow cytometer. Acquire data using the correct channel.