GeneCopoeia™
Expressway to Discovery

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Product Information

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

Cat. Number	Label	Unit Size
L101A	Andy Fluor 350	50 µL
L101B	Andy Fluor 350	250 µL
L104A	Andy Fluor 405	50 µL
L104B	Andy Fluor 405	250 µL
L107A	Andy Fluor 430	50 µL
L107B	Andy Fluor 430	250 µL
L110A	Andy Fluor 488	50 µL
L110B	Andy Fluor 488	250 µL
L114A	Andy Fluor 555	50 µL
L114B	Andy Fluor 555	250 µL
L117A	Andy Fluor 568	50 µL
L117B	Andy Fluor 568	250 µL
L120A	Andy Fluor 594	50 µL
L120B	Andy Fluor 594	250 µL
L126A	Andy Fluor 647	50 µL
L126B	Andy Fluor 647	250 µL
L129A	Andy Fluor 680	50 µL
L129B	Andy Fluor 680	250 µL
L132A	Andy Fluor 750	50 µL
L132B	Andy Fluor 750	250 µL
L135A	Cy3	50 µL
L135B	Cy3	250 µL
L138A	Cy5	50 µL
L138B	Cy5	250 µL
L141A	Cy5.5	50 µL
L141B	Cy5.5	250 µL
L144A	Cy7	50 µL
L144B	Cy7	250 µL
L147A	FITC	50 µL
L147B	FITC	250 µL
L150A	Biotin	50 µL
L150B	Biotin	250 µL
L153A	HRP	50 µL
L153B	HRP	250 µL

Storage upon receipt:
- **4 °C or -20 °C**
- **Protect from light**
- **Avoid freeze-thaw cycles**

Product Description

GeneCopoeia offers an extensive line of goat anti–rabbit IgG conjugates labeled with a wide selection of fluorescent dyes, biotin, or HRP.

Fluorescent Goat Anti–Rabbit IgG conjugates are ideal for fluorescence microscopy and confocal laser scanning microscopy, flow cytometry, and fluorescent western detection. The breadths of fluorescent markers we offer allow our reagents to be tailored to almost any fluorescent detection system.

The Goat Anti–Rabbit IgG antibody conjugates are prepared from affinity purified antibodies that react with IgG heavy chains and all classes of immunoglobulin light chains from rabbit. To minimize cross-reactivity, the goat anti–rabbit 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 or biotin 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.

Guidelines for Use

The Goat Anti–Rabbit IgG antibody conjugates are prepared at 2 mg/mL in 0.1 M sodium phosphate, 0.1 M NaCl, 5 mM sodium azide, pH 7.5.

Centrifuge the 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.