



**Andy Fluor™ 594 Goat Anti-Rabbit IgG (H+L) Antibody**

**SKU#: VB-L120B**

**Description**

Andy Fluor™ 594 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 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.

**Specification**

Physical State	Lyophilized powder	Host/Isotype	Goat
Purification	Immunoaffinity chromatography	Class & Form	Polyclonal/ Whole Antibody
Buffer	PBS, pH 7.4	Type	Secondary Antibody
Preservative	0.02% sodium azide	Tested Species Reactivity	Rabbit
Stabilizer	0.1% BSA	Target Class	IgG (H+L)
Label	Andy Fluor™ 594	Excitation/Emission	590/615 nm

**Package Size**

500 µg (Lyophilized powder)

**Reconstitution and Storage:**

Store lyophilized powder at 2-8°C. When ready to use, rehydrate with dH2O (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 2-8°C 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 °C 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-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  $1 \times 10^6$  cells should yield satisfactory result.

## General Protocols

### Immunofluorescence Protocol for Microscopy

#### 1. Preparation of Slides

##### For Cultured Cells

- Grow cultured cells on sterile glass cover slips or slides overnight at 37 ° C
- Wash briefly with PBS
- Fix as desired. Possible procedures include: a) 20 minutes with 10% formalin in PBS (keep wet); 2) 10 minutes with ice cold methanol, allow to air dry; 3) 10 minutes with ice cold acetone, allow to air dry
- Wash in PBS

##### For Frozen Sections

- Snap frozen fresh tissues in liquid nitrogen or isopentane pre-cooled in liquid nitrogen, embedded in OCT compound in cryomolds. Store the frozen tissue block at -80°C until ready for sectioning.
- Transfer the frozen tissue block to a cryotome cryostat (e.g. -20°C) prior to sectioning and allow the temperature of the frozen tissue block to equilibrate to the temperature of the cryotome cryostat.
- Section the frozen tissue block into a desired thickness (typically 5-10 µm) using the cryotome.
- Place the tissue sections onto glass slides suitable for immunohistochemistry (e.g. Superfrost).
- Sections can be stored in a sealed slide box at -80°C for later use.
- Before staining, warm slides at room temperature for 30 minutes and fix in ice cold acetone or ice cold methanol for 10 minutes. Air dry for 30 minutes.
- Wash in PBS

##### For Paraffin Sections

- Deparaffinize sections in xylene, 3×5min.
- Hydrate with 00% ethanol, 2×2min.
- Hydrate with 95% ethanol, 2×2min.
- Rinse in distilled water.
- Follow procedure for pretreatment as required.

#### 2. Antigen retrieval

Most formalin-fixed tissues require an antigen retrieval step before immunohistochemical staining can proceed. Heat-mediated and enzymatic antigen retrievals are common methods.

- **For Citrate:** Bring slides to a boil in 10 mM sodium citrate buffer, pH 6.0; maintain at a sub-boiling temperature for 10 minutes. Cool slides on bench top for 30 minutes.
- **For EDTA:** Bring slides to a boil in 1 mM EDTA, pH 8.0; follow with 15 minutes at a sub-boiling temperature. No cooling is necessary.
- **For TE:** Bring slides to a boil in 10 mM TE/1 mM EDTA, pH 9.0; then maintain at a sub-boiling temperature for 18 minutes. Cool at room temperature for 30 minutes.
- **For Pepsin:** Digest for 10 minutes at 37°C.

*Note: Generally it is not necessary to pretreat frozen sections or cultured cells with above antigen retrieval protocol.*

### 3. Staining Procedure

- Rinse sections in PBS-Triton X-100 (0.025%) for 2×2min
- **Serum Blocking:** incubate sections with 3-4 drops of RTU normal horse serum for 30 minutes to block non-specific binding of immunoglobulin.
- **Primary Antibody:** incubate sections with primary antibody (rabbit or rabbit IgG) at appropriate dilution in antibody dilution buffer (SKU#: VB-6002) for 1-2 hour at room temperature or overnight at 4 °C. Rinse in PBS.
- **Detection:** incubate sections with 3-4 drops of RTU Andy Fluor™ 594 Goat Anti-Rabbit IgG conjugates for 30 minutes at room temperature.
- Rinse in PBS for 3×2min.
- Count stain with Dapi (blue fluorescence) or other fluorescence nuclei stain dye (red color).
- Mount with aqueous mounting medium
- The slides can be **visualized** under fluorescence microscope using the correct filter.
- 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  $1 \times 10^6$  cells into  $12 \times 75$  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  $\mu\text{g/mL}$  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.