



**VitroView™ FL594 Antibody Labeling Kit
(Red, for 2×100 µg antibody)**

SKU: VB-6401

Description

The VitroView™ FL594 Antibody Labeling Kit provides a highly efficient workflow for conjugating IgG antibodies with FL594 dye. The kit utilizes amine-reactive NHS esters to form a stable, covalent bond with the antibody's primary amines. Ready-to-use dye-removal columns are included to separate the labeled antibody from excess unreacted fluorophore, consistently achieving final yields above 90%.

Key Advantages

- High Signal-to-Noise Ratio: Deliver superior results with minimal background fluorescence and reduced non-specific binding.
- Rapid & User-Friendly: Optimized protocol ensures minimal hands-on time and highly reproducible results.
- Complete System: Contains essential primary reagents required for successful fluorophore conjugation.
- Streamlined Workflow: Simple, direct steps minimize potential handling errors and save valuable bench time.

Specifications

Product	Molecular Weight	Ex/Em	Molar extinction coefficient (cm ⁻¹ M ⁻¹)	CF ₂₈₀
FL594	819.9	590/617	92,000	0.04

Kit Contents

SKU	Contents	Amount
VB-6401-1	FL594 reactive dye	2 vials
VB-6401-2	Reactive buffer	50 mL
VB-6401-3	Free dye removal column	2 Unit

Storage

Store reactive dye at -20 °C immediately upon receipt.

Reagents and Materials Required but Not Provided

- Microcentrifuge capable of 5,000 × g
- 10 mM phosphate-buffered saline (PBS), pH 7.4
- Amicon Ultra-4 Centrifugal Filter (Millipore)

Protocol

1. Prepare your antibody before using a labeling kit.

1) Pre-Labeling Requirements

- Purity: Antibody must be >90% pure.
- Concentration: Ideal range is 0.5–2.0 mg/mL.
- Formulation: Must be completely free of amine-containing additives and carrier proteins.

2) Common Interfering Substances

Substance	Maximum Allowed	Why It Interferes	Solution
Tris / Glycine	0 mM	Competes for binding sites	Dialysis / Buffer exchange
Sodium Azide	<0.02% (Ideally 0%)	Inhibits certain reactions	Dialysis / Buffer exchange
BSA / Gelatin	0%	Labels the carrier protein instead	Protein A/G or other affinity purification
Glycerol	<10%	Reduces labeling efficiency	Dialysis / Buffer exchange
Ammonium ions	0 mM	Competes for reactive groups	Dialysis / Buffer exchange

3) Dialysis or buffer exchange your antibody if your antibody contains Tris / Glycine, Sodium Azide, Glycerol or Ammonium ions, we suggest use a centrifugal spin filter (e.g., Amicon Ultra).

- Choose an appropriate Molecular Weight Cut-Off (MWCO): For IgG (150 kDa): Use a 10 kDa or 30 kDa MWCO filter.
- Add 500 µL of compatible buffer (e.g., 1X PBS, pH7.4).
- Spin at 14,000 × g for 5 minutes.
- Discard the flow-through.
- Load the antibody sample into the filter.
- Top up with PBS to the maximum volume line.
- Spin according to manufacturer speed recommendations.
- Discard flow-through; repeat this wash step 3 times.
- Invert the spin column into a clean collection tube.
- Spin at 1,000 × g for 2 minutes to collect the purified antibody.
- Concentration Verification
 - UV Absorbance: Measure at A280 using a spectrophotometer.
 - IgG Extinction Coefficient: Use 1.4 for a 1 mg/mL solution.
 - Calculation: Concentration (mg/mL) = A280 / 1.4.

4) Purify your antibody if your antibody contains BSA / Gelatin

To separate BSA or gelatin from your antibody, you must use a method based on chemical properties or affinity rather than simple size-exclusion filtration:

- Negative Selection Resins: Commercial kits like the Thermo Scientific Melon Gel IgG Purification System bind to BSA and gelatin while allowing the pure antibody to flow straight through.
- Dedicated BSA Removal Kits: Specialized kits such as the Abcam BSA Removal Kit (ab173231) chemically precipitate or capture BSA out of the solution specifically for downstream conjugation.
- Protein A, G, or L Affinity Chromatography: This method binds your antibody tightly to a resin while you wash the BSA and gelatin away, allowing you to elute the pure antibody afterward.

- 5) Adjust the antibody concentration to 1 mg/mL. If the antibody concentration is ≥ 1 mg/mL, dilute it to 1 mg/mL; if it is < 1 mg/mL, concentrate the sample using an appropriate centrifugation method until it reaches 1 mg/mL. Add one-tenth volume of reaction buffer to the antibody solution.
 - 6) For Lyophilized Antibody: Dilute the provided Reactive Buffer 10-fold with deionized water. Reconstitute the lyophilized antibody powder in this diluted buffer to achieve a final concentration of 1 mg/mL.
2. Antibody Labeling Reaction
 - 1) Transfer 100 μ L of the prepared 1 mg/mL antibody solution directly into one vial of FL488 reactive dye.
 - 2) Cap the vial securely and gently invert it several times to completely dissolve and mix the dye with the antibody.
 - 3) Incubate the reaction mixture for 1 hour at room temperature in the dark.
 3. Free Dye Removal & Purification
 - 1) Remove the column cap and pour off the storage buffer. Cut the sealed bottom end of the column at the marked notch.
 - 2) Equilibrate the column: Fill the column with PBS (pH 7.4), allowing the buffer to gravity-flow completely through the packed bed. Repeat this step 4 times, discarding all flow-through.
 - 3) Dilute the reaction mixture: Add PBS to your 100 μ L labeled antibody solution to bring the final volume up to 2.5 mL.
 - 4) Load the 2.5 mL sample carefully onto the top of the equilibrated column bed.
 - 5) Allow the sample to completely enter the packed bed by gravity flow. Discard the resulting flow-through.
 - 6) Elute the purified antibody: Place a clean collection tube underneath the column and add 3.5 mL of PBS buffer to elute the conjugate.
 4. Optional: Antibody Concentration

If required, concentrate the eluted antibody to the desired working concentration using a 10-kDa or 30-kDa molecular-weight cutoff Amicon[®] centrifugal filter unit (not provided).
 5. Optional: efficiency test of labeling reaction
 - 1) Calculate antibody concentration C (mg/ml) and dye/antibody degree of labeling (DOL) by measuring OD₂₈₀ and OD₅₉₀ absorbance:

$$C = (MW_{Ab} \times [OD_{280} - CF_{280} \times OD_{590}]) / \epsilon_{Ab}$$

$$DOL = (OD_{590} \times \epsilon_{Ab}) / (\epsilon_{dye} \times [OD_{280} - CF_{280} \times OD_{590}])$$

MW_{Ab}: Molecular weight of antibody (150 kDa)

OD₂₈₀: Absorbance at 280nm of the dye-antibody conjugate

OD₅₉₀: Absorbance at 590nm of dye

CF₂₈₀: Correction factor at 280nm accounting for absorbance of dye at 280nm (0.04)

ϵ_{Ab} : Molar extinction coefficient at 280nm of antibody (210,000 cm⁻¹M⁻¹)

ϵ_{dye} : Molar extinction coefficient at 590nm of the dye (92,000 cm⁻¹M⁻¹)

- 2) Make final concentration at 1mg/ml in PBS buffer with 0.1% BSA, 0.02% Na₃N (option).

Note: This product is intended strictly for laboratory research purposes. It is not approved for therapeutic, clinical, or diagnostic procedures in humans or animals.

Precautions: Handle with care. Avoid contact with eyes, skin, and clothing. Do not ingest. Wear gloves.