VitroView™ FL647 Antibody Labeling Kit
(Far Red, for 2×100 µg antibody)

SKU: VB-6402

Description

The VitroView™ FL647 Antibody Labeling Kit provides a highly efficient workflow for conjugating IgG antibodies with FL647 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}
FL647	1022	648/671	270,000	0.04

Kit Contents

SKU	Contents	Amount
VB-6402-1	FL647 reactive dye	2 vials
VB-6402-2	Reactive buffer	50 uL
VB-6402-3	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).
   i) Choose an appropriate Molecular Weight Cut-Off (MWCO): For IgG (150 kDa): Use a 10 kDa or 30 kDa MWCO filter.
   ii) Add 500 µL of compatible buffer (e.g., 1X PBS, pH7.4).
   iii) Spin at 14,000 × g for 5 minutes.
   iv) Discard the flow-through.
   v) Load the antibody sample into the filter.
   vi) Top up with PBS to the maximum volume line.
   vii) Spin according to manufacturer speed recommendations.
   viii) Discard flow-through; repeat this wash step 3 times.
   ix) Invert the spin column into a clean collection tube.
   x) Spin at 1,000 × g for 2 minutes to collect the purified antibody.
   xi) 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 1 mg/mL is reached. 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 µL 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 µL 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 OD_{280} and OD_{650} absorbance:

$C = (MW_{Ab} \times [OD_{280} - CF_{280} \times OD_{650}]) / \varepsilon_{Ab}$
$DOL = (OD_{650} \times \varepsilon_{Ab}) / (\varepsilon_{dye} \times [OD_{280} - CF_{280} \times OD_{650}])$

MW_{Ab}: Molecular weight of antibody (150 kDa)
OD_{280}: Absorbance at 280nm of the dye-antibody conjugate
OD_{650}: Absorbance at 650nm of dye
CF_{280}: Correction factor at 280nm accounting for absorbance of dye at 280nm (0.04)
ε_{Ab}: Molar extinction coefficient at 280nm of antibody (210,000 $cm^{-1}M^{-1}$)
ε_{dye}: Molar extinction coefficient at 650nm of the dye (270,000 cm^{-1}M-1)

2) Make final concentration at 1mg/ml in PBS buffer with 0.1% BSA, 0.02% Na_3N (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.