SAIVI™ Rapid Antibody Labeling Kits

Table 1. Contents and storage information.

Material	Amount	Storage	Stability
Sodium bicarbonate (Component A)	3 vials	• 2–6˚C • Do not freeze • Protect from light	When stored as directed the kit is stable for at least 3 months
Regulator solution* (Component B)	1 vial		
Alexa Fluor® reactive dye* (Component C)	3 vials		
Purification columns† (Component D)	3 columns		
Purification resin (size exclusion resin in PBS, pH 7.2, Component E)	3 bottles each		
Phosphate buffered saline (PBS, pH 7.4), sterile (Component F)	50 mL		
Syringe, 1 mL, sterile (Component G)	3 syringes		
Syringe filters, 13 mm, 0.2 µm (Component H)	3 filters		
Column loading pipettes (Component I)	3 pipettes		
Catch tubes (Component J)	15 tubes		

* The regulator solution and the Alexa Fluor® reactive dye components are specific to the kit purchased. Do not swap these components between Cat. no. S30045 and S30046. † Each purification column has a frit, a tip cap, and a top funnel. NA = Not applicable.

Number of assays: Each kit provides materials for 3 labeling reactions using 0.5–3 mg of protein per reaction.

Approximate fluorescence excitation and emission maxima: ~679/702 nm for Alexa Fluor® 680 dye and 749/775 nm for Alexa Fluor® 750 dye.

Introduction

Invitrogen's Molecular Probes™ SAIVI™ Antibody Labeling Kits provide a convenient means to label antibodies with the near-infrared Alexa Fluor® 680 or Alexa Fluor 750 dyes. The conjugation protocol supplied here produces an optimal degree of labeling for *in vivo* imaging applications (DOL; ~2) over a 6-fold antibody concentration range with no adjustments in reaction volume, dye concentration, or antibody concentration necessary (Figure 1). The water-soluble NIR Alexa Fluor® reactive dyes are supplied lyophilized and ready to use. No organic solvents are used in the labeling protocol, and purification of the dye-labeled conjugate is achieved with a rapid, simple protocol completed in less than 10 minutes, with excellent reproducibility and using azide-free buffers. Using this procedure, optimally labeled antibodies are ready for applications that require azide-free reagents, such as live-cell imaging or direct injection into animals.

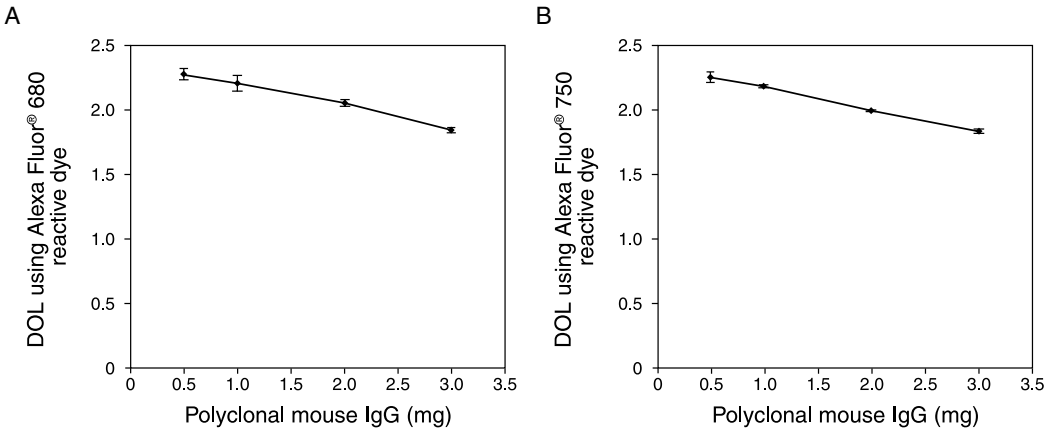

Figure 1. Four different amounts of mouse polyclonal IgG were labeled using the SAIVI™ Alexa Fluor® 680 Antibody Labeling Kit (panel A) or the SAIVI™ Alexa Fluor® 750 Antibody Labeling Kit (panel B). Over this 6-fold protein concentration range, the DOL obtained using these kits was ~2, which is appropriate for *in vivo* imaging applications.

Experimental Protocol

Protein Preparation

The antibody solution should be free of ammonium ions, primary amines or contaminating polypeptides and proteins. If the antibody is in or has been lyophilized from an unsuitable buffer (such as Tris or glycine) or purified with ammonium sulfate the buffer must be replaced with 1X phosphate buffered saline (PBS) by dialysis or gel filtration. The presence of low concentration of sodium azide (≤3 mM) or thimerosal (≤1 mM) will not interfere with the conjugation reaction.

Conjugation Reaction

Reactions are performed at room temperature (18–26˚C). All solutions, including the antibody solution, should be equilibrated to room temperature before use.

1.1 Prepare a solution of sodium bicarbonate, pH 8.3, by adding 1 mL of deionized water to the provided vial of sodium bicarbonate (Component A). Dissolve completely by vortexing. This solution is stable for 1 week at 4˚C.

1.2 Combine in a 1.5 mL microcentrifuge tube:

50 µL sodium bicarbonate buffer (from step 1.1)
10 µL regulator solution (Component B)
500 µL antibody solution (1 mg/mL to 6 mg/mL)

1.3 Transfer the mixture (step 1.2) to a reaction vial containing the lyophilized Alexa Fluor® reactive dye (Component C). Dissolve the dye completely by pipetting the solution repeatedly.

1.4 Incubate the antibody/dye solution (step 1.3) for 60 minutes at room temperature and protected from light.

Note: It is not necessary to stir, shake, or mix the reaction vial during the incubation.

Preparing the Purification System

2.1 Fit the funnel onto the top of one of the purification columns (Component D).

2.2 Suspend the purification resin (Component E) by repeated inversion of the capped bottle.

2.3 Remove the tip cap from the purification column and pour the suspension into the column. Allow the resin to settle as the buffer drains.

2.4 Add 12 mL of PBS (Component F) to the bottle, suspend residual resin, and pour it into the column. Allow the PBS to drain completely.

Note: Be sure the column has completely drained by gravity and has stopped dripping before loading the antibody/dye mixture. The column will remain hydrated for several minutes after the column has drained.

Note: The column funnel can be removed at this time for better access to the resin bed during sample loading.

Purifying the Fluorescent Conjugate

Note: It is not necessary to mix, shake, or vortex the sample before it is loaded onto the column.

3.1 At the end of the 60 minute conjugation reaction, gently layer the reaction mixture (from step 1.4) on the top of the column bed using a clean column loading pipette (Component I).

3.2 Allow the reaction mixture to absorb into the column bed. Ensure the column has completely stopped dripping before proceeding to the next step.

3.3 Gently layer 1.3 mL of PBS onto the column bed. Ensure the column has stopped dripping before proceeding to the next step.

Note: The antibody-dye conjugate will move as a diffuse band away from the intensely colored unincorporated dye and will enter the frit area and dead space just above the tip.

3.4 Apply 900 µl PBS to the column and collect the eluate in a clean, 1.5 ml catch tube (Component J) or appropriate equivalent. The dye-conjugated protein will be a light-to-medium blue liquid (Alexa Fluor® 680 conjugates) or blue-green liquid (Alexa Fluor® 750 conjugates). The unincorporated dye will remain on the column as a broad, intensely colored band.

3.5 Remove the plunger from one of the 1 mL syringes (Component G).

3.6 Fit one of the 0.2 µm syringe filters (Component H) to the end of the syringe.

3.7 Pipet the dye-antibody eluate (from step 3.4) into the syringe.

3.8 Replace the plunger and filter the eluate into an appropriate sterile tube using one smooth movement.

Note: When used in mice as an imaging reagent, typically 50–100 µg of labeled antibody is injected intravenously per animal. This amount will vary, however, depending on the experimental model and application.

Determining the Degree of Labeling (DOL)

Determine the Peak Absorbances of the Purified Conjugate

The eluted conjugate should be mixed thoroughly. Dilute a sample of the purified conjugate with PBS 1:10 or 1:20 and measure the protein absorbance at 280 nm (A_{280}) and dye at 679 nm (A_{679} for Alexa Fluor® 680 conjugates) or 752 nm (A_{752} for Alexa Fluor® 750) conjugates or determine peak protein and dye absorbances by scanning absorbance.

Calculate DOL

DOL can be calculated using the formula below. Use the correction factors (CF) for fluorophore absorbance at 280 nm and the molar extinction coefficients (ε):

IgG: $\varepsilon = 203{,}000\ M^{-1}cm^{-1}$ at 280 nm

Alexa Fluor® 680 dye:

CF: 0.05
$\varepsilon = 180{,}000\ M^{-1}cm^{-1}$ at 679 nm

Alexa Fluor® 750 dye:

CF: 0.034
$\varepsilon = 270{,}000\ M^{-1}cm^{-1}$ at 752 nm

For Alexa Fluor® 680 dye conjugates:

$$\text{Protein concentration (M)} = \frac{[A_{280}-(A_{679}\times 0.05)]\times \text{dilution factor}}{203{,}000}$$

$$\text{Moles of dye/mole of protein (DOL):}\ \frac{A_{679}\times \text{dilution factor}}{(180{,}000\times \text{protein concentration (M)})}$$

For Alexa Fluor® 750 dye conjugates:

$$\text{Protein concentration (M)} = \frac{[A_{280}-(A_{752}\times 0.034)]\times \text{dilution factor}}{203{,}000}$$

$$\text{Moles of dye/mole of protein (DOL):}\ \frac{A_{752}\times \text{dilution factor}}{(270{,}000\times \text{protein concentration (M)})}$$

Product List **Current prices may be obtained from our website or from our Customer Service Department.**

Cat #	Product Name	Unit Size
S30045	SAIVI™ Rapid Antibody Labeling Kit, Alexa Fluor® 680 *3 labelings*	1 kit
S30046	SAIVI™ Rapid Antibody Labeling Kit, Alexa Fluor® 750	1 kit

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