Revised: February 18, 2019

Product Information

Mix-n-Stain™ Nanobody Labeling Kits

Unit Size: One labeling reaction per kit

Kit Contents

Component	5-20 ug labeling	20-50 ug labeling
Dye or biotin vial*	1 vial Component A	1 vial Component A
Mix-n-Stain™ Reaction Buffer, 10X	15 uL 99951-1	15 uL 99951-1
BSA Solution (1 mg/mL)**	100 uL 99856-100uL	100 uL 99856-100uL
Mix-n-Stain™ Quencher, 10X	15 uL 99854-15uL	15 uL 99854-15uL
Ultrafiltration vial (MWCO=3K)	1 each 99985	1 each 99985

* Mix-n-Stain™ dye or biotin is supplied as a lyophilized solid. The amount in the vial is very small and usually is not visible until solution is added; biotin and CF®405S remain colorless in solution. See FAQs (page 3) for more information.

** BSA Solution is provided for optional adjustment of protein input. See Kit Compatibility & Protocol Selection Note 3c for more information.

Storage and Handling

Store kit at -20°C. Kit components are stable for at least 12 months from date of receipt when stored as recommended.

Catalog Numbers & Spectral Properties

Label/Dye	Ex/Em	Labeling size/Catalog number	
		5-20 ug	20-50 ug
Biotin	N/A	92500	92501
CF®405S	404/431 nm	92502	92503
CF®488A	490/515 nm	92504	92505
CF®568	562/583 nm	92506	92507
CF®594	593/614 nm	92508	92509
CF®640R	642/662 nm	92510	92511
CF®647	650/665 nm	92512	92513
CF®680R	680/701 nm	92514	92515

Product Description

These kits are designed for labeling a single-domain Nanobody® (also called camelid single variable or VHH domains) with Biotium's bright and photostable CF® dyes or biotin. The kits allow labeling of 5-50 ug nanobody in just 30 minutes, with minimal hands-on time and no purification. Labeling tolerates common buffer additives, including BSA.

Simply mix your nanobody with the reaction buffer and pre-measured dye provided, followed by a brief incubation. Any free dye or label is no longer reactive at the end of the labeling, so the conjugate is ready for staining without further purification. The conjugate will be labeled with an average of 1-2 dye or biotin molecules per nanobody molecule. Mix-n-Stain™ labeling is covalent, so labeled nanobodies can be used for multiplex staining without transfer of dye or biotin between proteins.

See page 3 for frequently asked questions (FAQs).

Considerations for Staining with Mix-n-Stain™ Labeled Nanobodies

Direct immunofluorescence staining should be done with high affinity nanobodies against abundant targets. Nanobodies should be validated using secondary detection before performing direct labeling.

Tissue staining by direct immunofluorescence can be challenging due to tissue autofluorescence and target integrity issues in human tissue. See our TrueBlack® line of background reducers (Related Products) for reducing background in tissue sections and other samples. We also offer CF® Dye Tyramide Signal Amplification Kits, which can be used to amplify immunofluorescence signal to improve signal in tissue staining.

Kit Compatibility & Protocol Selection

1. Mix-n-Stain™ Nanobody Labeling Kits are optimized for labeling single-chain nanobodies. Mix-n-Stain™ Antibody Labeling Kits (see page 3) should be used for labeling IgG antibodies.
2. The kits are optimized for a single labeling reaction. We do not recommend trying to split the kit for more than one labeling.
3. The kits come in two sizes, for labeling 5-20 ug or 20-50 ng of nanobody.

   a. If the nanobody is in PBS with no other protein added, use the kit size that spans the amount of nanobody you wish to label. For labeling 20 ug, we recommend using the 5-20 ug kit.

   b. If the nanobody buffer contains BSA or gelatin, choose the kit size that matches the amount of total protein in the labeling reaction. If BSA is present, both nanobody and BSA will be labeled. Labeled BSA has minimal effect on immunofluorescence background, because it is removed during washing. Labeling should not be done with BSA if the nanobody will be used in other applications where labeled BSA may interfere.

   c. The kits are optimized for labeling nanobody amounts at the upper end of the kit range (20 ug for the 5-20 ug kit, or 50 ug for the 20-50 ug kit). Labeling nanobody amounts at the lower end of the kit range also will work, but may result in over-labeling, which can reduce nanobody binding affinity and fluorescence signal. For labeling lower amounts of nanobody, adding BSA to adjust the total protein amount to the upper end of kit range will result in more optimal labeling. If BSA is present in the reaction, it also will be labeled; see Note 3b for more information.
4. See Table 1 for a list of compatible buffer components. A microcentrifuge ultrafiltration vial is provided in the kit to rapidly remove incompatible small molecules (<3 kDa). The ultrafiltration vial will not remove stabilizer proteins.
5. The optimal protein concentration for labeling is 1 mg/mL. If the nanobody solution is more dilute, use the ultrafiltration vial to concentrate it.

Mix-n-Stain™ nanobody labeling overview

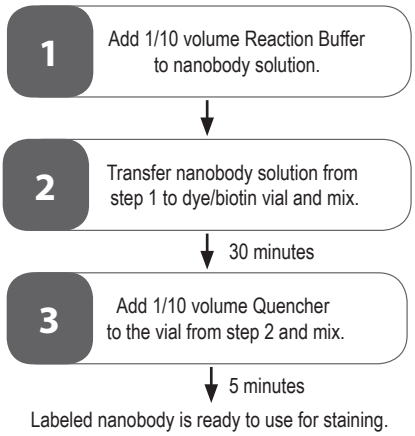

Table 1. Labeling Compatibility and Protocol Selection

Component	Compatibility
Sodium Azide	Compatible
Glycerol	≤10%: Compatible >10%: Perform ultrafiltration (Section A)
Tris or Glycine	Perform ultrafiltration (Section A)
BSA or gelatin	Use the modified labeling protocol (Section C) See Kit Compatibility & Protocol Selection Notes 3b & 3c.
Nanobody concentration less than 1 mg/mL	Perform ultrafiltration and resuspend at 1 mg/mL in PBS before labeling.
Nanobody ug amount at lower end of kit range	Optional: Add BSA (provided) to adjust the protein ug amount to the upper end of the kit range. Follow the modified labeling protocol (Section C). See Kit Compatibility & Protocol Selection Notes 3b & 3c.

A. Ultrafiltration Protocol

Important: Before you begin, use Table 1 to determine whether your nanobody requires ultrafiltration before labeling. If necessary, contact the manufacturer of your nanobody to find out the concentration of nanobody and stabilizers. If your nanobody does not require ultrafiltration, proceed to the appropriate labeling protocol (see Table 1).

The ultrafiltration membrane has a molecular weight cut-off of 3,000. Therefore, molecules smaller than 3 kDa will flow through the membrane, and molecules larger than 3 kDa, including nanobodies and any stabilizer proteins, will be retained on the upper surface of the membrane (Figure 1). Take care not to touch the membrane with pipette tips, which could tear or puncture the membrane, resulting in loss of nanobody. Additional ultrafiltration vials also can be purchased separately (cat. no. 22018).

Note: Repeated filtration of large sample volumes (~500 uL) can lead to membrane failure. We recommend keeping sample volumes at or below 350 uL.

Ultrafiltration Vial Capacities:
Maximum Sample Volume: 500 uL (see note above)
Final Concentrate Volume: 15 uL
Filtrate Receiver Volume: 500 uL
Hold-up Volume (Membrane/Support): < 5 uL

1. Add an appropriate amount of nanobody to the membrane of the ultrafiltration vial, being careful not to touch the membrane. Centrifuge the solution at 14,000 x g in a microcentrifuge for one minute. Check to see how much liquid has filtered into the filtrate collection tube (lower chamber). Repeat the centrifugation until all of the liquid has filtered into the collection tube. Discard the liquid in the collection tube.
2. For nanobody concentration, proceed to Step 3. For clean-up, add an equal volume of 1X PBS to the membrane. Centrifuge the vial at 14,000 x g until the liquid has filtered into the filtrate receiving tube.
3. Add an appropriate concentration of PBS to the membrane to obtain a final protein concentration of 1 mg/mL. Carefully pipet the PBS up and down over the upper surface of the membrane to recover and resuspend the nanobody.
4. Transfer the recovered nanobody solution to a fresh microcentrifuge tube.
5. Proceed to the appropriate labeling protocol (see Table 1).

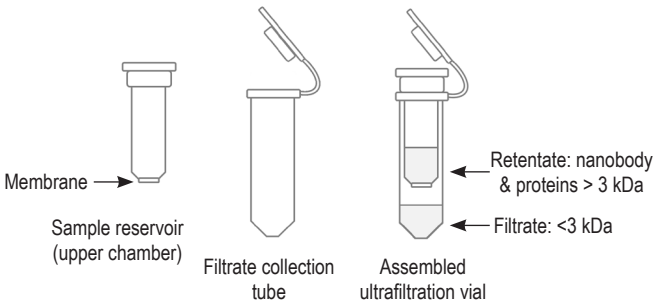

Figure 1. 3K MWCO ultrafiltration vial components.

B. Standard Labeling Protocol

For labeling nanobodies with no BSA or other stabilizer

1. Warm up the Mix-n-Stain™ Reaction Buffer and Quencher vials to room temperature and vortex to mix before use. Centrifuge the vials briefly to collect the solutions at the bottom of the vials.
2. Start with your nanobody at 1 mg/mL in PBS. Transfer an appropriate ug amount of nanobody (within the ug range of your kit) to a clean tube for labeling.
3. Add the 10X Mix-n-Stain™ Reaction Buffer to the nanobody solution at a ratio of 1:10 so that the solution contains a final concentration of 1X Reaction Buffer (for every 9 uL of nanobody solution, add 1 uL of 10X Reaction Buffer). Mix completely by pipetting up and down or gentle vortexing. **Note:** Adding Reaction Buffer is not optional. Labeling will not occur without it.
4. Transfer the entire solution from Step 3 to the vial containing lyophilized dye or biotin (Component A). There is no need to measure the amount of the dye biotin in the vial. Vortex the vial for a few seconds.
5. Incubate the vial in the dark for 30 minutes at room temperature. Incubating for longer times won't affect the labeling.
6. Add the 10X Mix-n-Stain™ Quencher to the reaction vial at a ratio of 1/10 (1 uL of Quencher for every 9 uL of solution from step 3) and mix.
7. Incubate 5 minutes in the dark at room temperature. The nanobody is now ready to use for staining. The concentration of labeled nanobody is the starting ug amount of nanobody divided by the total volume.

C. Modified Labeling Protocol

For labeling nanobodies with BSA or other stabilizer protein added. See Kit Compatibility & Protocol Selection Notes 3b & 3c.

1. Warm up the Mix-n-Stain™ Reaction Buffer, BSA (optional), and Quencher vials to room temperature and vortex to mix before use. Centrifuge the vials briefly to collect the solutions at the bottom of the vials.
2. Optional: If your nanobody amount is at the lower end of the kit range, add the appropriate amount of the provided BSA solution to bring the total amount of protein to the upper end of the kit range. For example, if you wish to label 5 ug of nanobody with the 5-20 ug kit, add 15 uL of 1 mg/mL BSA to 5 ug of nanobody bring the protein amount to 20 ug total.
3. Start with your nanobody + BSA at 1 mg/mL (total protein concentration) in PBS. Transfer an appropriate ug amount of total protein (within the range of your kit) to a clean tube for labeling.
4. Mix the 10X Mix-n-Stain™ Reaction Buffer with the protein solution at a ratio of 1:10 so that the solution contains a final concentration of 1X Reaction Buffer (for every 9 uL of protein solution, add 1 uL of 10X Reaction Buffer). Mix completely by pipetting up and down or gentle vortexing.
Note: Adding Reaction Buffer is not optional. Labeling will not occur without it.
5. Transfer the entire solution from Step 4 to the vial containing the lyophilized dye or biotin (Component A). There is no need to measure the amount of the dye or biotin in the vial. Vortex the vial for a few seconds.
6. Incubate the vial in the dark for 30 minutes at room temperature. Incubating for longer times won't affect the labeling.
7. Add the 10X Mix-n-Stain™ Quencher to the reaction vial at a ratio of 1/10 (1 uL of Quencher for every 9 uL of solution from step 4) and mix.
8. Incubate 5 minutes in the dark at room temperature. The nanobody is now ready to use for staining. The concentration of labeled nanobody is the starting ug amount of nanobody divided by the total volume.

Storage of Labeled Nanobodies

We recommend adding 2 mM sodium azide to labeled nanobodies for storage at 4°C. Nanobodies also can be stored in single use aliquots at -20°C. Store fluorescent dye conjugates protected from light. Conjugates should be stable for at least 6 months when stored as recommended.

Frequently Asked Questions (FAQs)

Question	Answer
What are CF® dyes?	CF® dyes are highly water soluble, small organic dyes for labeling proteins and nucleic acids. CF® dyes are designed to be brighter and more photostable than competing dyes.
How do I remove the unconjugated free dye after labeling, since there is no purification step?	Because of the unique formulations of our dyes and labeling technology, it is not necessary to remove unconjugated free dye before staining. However, ultrafiltration can be used to remove free dye after labeling if desired.
Can I use Mix-n-Stain™ labeled nanobodies for multi-color staining, or will the dye transfer between proteins?	Mix-n-Stain™ labeling results in covalent linkage of dye and nanobody. At the end of the labeling reaction, no reactive dye remains, so there will be no transfer of dye to other proteins.
Can I use the kit for labeling proteins other than antibodies or nanobodies?	Mix-n-Stain™ Antibody Labeling Kits are optimized for labeling IgG antibodies, while Mix-n-Stain™ Nanobody Labeling Kits are optimized for labeling single-chain nanobodies. The kits could be used to label other proteins, but the number of dye molecules per protein molecule may not be optimal. Any conjugation method, including Mix-n-Stain™, may affect the biological activity of proteins. We also offer Mix-n-Stain™ CF® Dye Small Ligand Labeling Kits, for labeling amine-functionalized compounds such as SNAP-tag®, CLIP-tag™, and HaloTag® ligands, or other molecules (see Other Mix-n-Stain™ Labeling Kits).
What size kit should I use?	For nanobody labeling in the absence of stabilizer protein, use a kit range that matches the amount of antibody you wish to label. For labeling nanobody in the presence of stabilizer protein like BSA, choose a kit range that matches the total amount of protein in the sample that will be labeled. For labeling nanobody amounts at the lower end of the kit range, adding BSA to adjust the protein concentration can result in more optimal labeling. For details, see Kit Compatibility & Protocol Selection (page 1).
If my nanobody amount falls between the two kits, which one should I use?	We recommend using the smaller kit.
What dye/protein ratio should I use to ensure optimal labeling?	There is no need to measure the dye amount or vary the reaction time as long as the amount of your antibody to be labeled falls within the range specified for each kit.
Can I split the kit contents and use it more than one time?	No. The Mix-n-Stain™ kits are optimized for 1 labeling. We do not recommend that you try to split the kit to label for more than one reaction.
How important is the nanobody concentration in the labeling reaction?	The kits are optimized for labeling nanobodies at 1 mg/mL. Using higher or lower concentrations may result in either under- or over-labeling.
The Component A vial appears to be empty, should I ask for a replacement?	Mix-n-Stain™ dyes and labels are supplied as lyophilized solids. The amount of label in the vial is very small and usually is not visible. For green, red, and far-red dyes, the dye color will become visible when you mix your antibody solution into the vial. Biotin and CF®405S will appear colorless in solution.

Related Products

Catalog number	Product
22018	Ultrafiltration vial, 3K MWCO (pack of 5)
22004	Ultrafiltration vial, 10K MWCO (pack of 5)
30071	AccuOrange™ Protein Quantitation Kit
23012	TrueBlack® IF Background Suppressor System (Permeabilizing)
23013	TrueBlack® WB Blocking Buffer Kit
23007	TrueBlack® Lipofuscin Autofluorescence Quencher
40083	NucSpot® 470 Green Nuclear Counterstain
40061	RedDot™2 Far Red Nuclear Counterstain
23008	Drop-n-Stain EverBrite™ Mounting Medium
23005	CoverGrip™ Coverslip Sealant
22005	Mini Super^{HT} Pap Pen 2.5 mm tip, ~400 uses
22006	Super^{HT} Pap Pen 4 mm tip, ~800 uses
23006	Flow Cytometry Fixation/Permeabilization Kit
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative
22016	Permeabilization Buffer
22017	Permeabilization and Blocking Buffer
22020	10X Phosphate Buffered Saline (PBS) (4L Cubitainer®)
22013	Bovine Serum Albumin Fraction V
22014	Bovine Serum Albumin 30% solution
22010	10X Fish Gelatin Blocking Agent
22011	Fish Gelatin Powder

Other Mix-n-Stain™ Labeling Kits

Catalog number	Product
92230...92433	Mix-n-Stain™ CF® Dye Antibody Labeling Kits
92286,92266, 92244	Mix-n-Stain™ Biotin Antibody Labeling Kits
92294, 92295, 92296	Mix-n-Stain™ FITC Antibody Labeling Kits
92328, 92329, 92330	Mix-n-Stain™ Digoxigenin Antibody Labeling Kits
92325, 92326, 92327	Mix-n-Stain™ DNP Antibody Labeling Kits
92412...92418	Mix-n-Stain™ Cyanine Dye Antibody Labeling Kits
92404-92424	Mix-n-Stain™ Maxi 1 mg Scale Labeling Kits
92298-92299	Mix-n-Stain™ R-PE Antibody Labeling Kits
92306-92307	Mix-n-Stain™ APC Antibody Labeling Kits
92340-92341, 92346	Mix-n-Stain™ RPE-CF®647T Antibody Labeling Kits
92310-92311	Mix-n-Stain™ APC-CF®750T Antibody Labeling Kits
92300-92302	Mix-n-Stain™ HRP Antibody Labeling Kits
92314-92315	Mix-n-Stain™ AP Antibody Labeling Kits
92312-92313	Mix-n-Stain™ Glucose Oxidase Antibody Labeling Kits
92350-92364	Mix-n-Stain™ CF® Dye Small Ligand Labeling Kits

Please visit www.biotium.com to view our full selection of products featuring bright and photostable CF® dyes, including Mix-n-Stain™ Small Ligand Labeling Kits, primary & secondary antibodies, streptavidin, phalloidins, and much more.