Product Information

Mix-n-Stain™ Nanobody Thiol Labeling Kits

Unit Size: One labeling reaction per kit

Kit Contents

Component	5-20 ug Labeling	20-50 ug Labeling
Dye Vial*	1 vial Component A	1 vial Component A
Mix-n-Stain™ Reaction Buffer, 5X	99893 15 uL	99893 15 uL
Mix-n-Stain™ Activating Agent	99894 1 vial	99894 1 vial
Mix-n-Stain™ Quenching Buffer, 10X	99895 15 uL	99895 15 uL
Mix-n-Stain™ Storage Solution, 4X**	99896 15 uL	99896 15 uL

*Mix-n-Stain™ dye is supplied as a lyophilized solid. The amount in the vial is very small and usually not visible until solution is added.

**Mix-n-Stain™ Storage Solution contains recombinant BSA and >0.05% sodium azide.

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

Dye	Ex/Em	Labeling Size/Cat. No.	
		5-20 ug	20-50 ug
CF®488A	490/516 nm	92585	92586
CF®568	562/584 nm	92587	92588
CF®583R	585/609 nm	92589	92590
CF®594	593/615 nm	92591	92592
CF®647	652/668 nm	92593	92594
CF®680	681/698 nm	92595	92596
CF®740	742/767 nm	92597	92598

Product Description

Mix-n-Stain™ Nanobody Thiol Labeling Kits are designed for labeling a Nanobody® (VHH, also called camelid single domain antibody or SdAb), bearing a single cysteine (1x Cys), with Biotium's bright and photostable CF® Dyes. The kits allow labeling of either 5-20 ug or 20-50 ug of VHH within 2 hours in ambient conditions, and require minimal hands-on time and no purification.

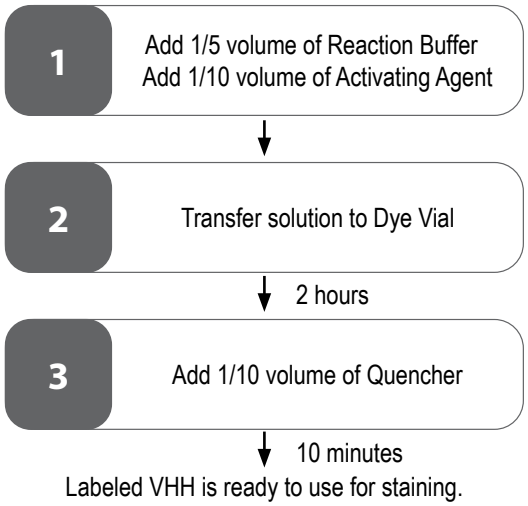
Simply mix your 1x Cys VHH with the provided reaction buffer, activating agent, and pre-measured dye, followed by a 2 hour incubation and brief quenching step. Any free dye or label is no longer reactive at the end of the labeling, so the conjugate is ready for staining without further purification. The expected conjugate will be labeled with a single dye molecule per 1x Cys VHH. Mix-n-Stain™ labeling is covalent, so labeled VHH can be used for multiplex staining without transfer of dye between targets.

Considerations for Staining with Mix-n-Stain™ Thiol Labeled VHH

Direct immunofluorescence staining should be done with high affinity VHH against abundant targets. VHH should be validated using secondary detection before performing direct labeling. Biotium offers CF® Dye secondary antibodies for detecting Alpaca VHH (see Related Products).

Tissue staining by direct immunofluorescence can be challenging due to tissue background fluorescence 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.

Mix-n-Stain™ Nanobody Thiol Labeling Kit Procedure Overview



Kit Compatibility

- Mix-n-Stain™ Nanobody Thiol Labeling Kits are optimized for labeling camelid single domain VHH. Mix-n-Stain™ Antibody Labeling Kits (see Other Antibody or Protein Labeling Kits) should be used for labeling IgG antibodies.
- The labeling reaction targets the cysteine amino acid residue and requires VHH constructs that are engineered to have an exposed cysteine residue (typically inserted at or near the N-terminus or C-terminus). Note that the native internal cysteine residues in VHH are disulfide-bonded and cannot be used for labeling. If your VHH does not contain an engineered free cysteine, our original Mix-n-Stain™ Nanobody Labeling Kits (see Other Antibody or Protein Labeling Kits) can be used to label VHH on lysine residues.
- The conjugation reaction has been optimized for VHH with a single, surface exposed cysteine residue, not two cysteines (i.e., 1x Cys, not 2x Cys). VHH with 2x Cys will be labeled, but the ratio of dye to cysteine residues will not be optimal and unlabeled exposed cysteine residues will still be present in the conjugate.
- The kits are optimized for a single labeling reaction. We do not recommend trying to split the kit for more than one labeling.
- The kits come in two sizes, for labeling 5-20 ug or 20-50 ug of VHH.
    - If the VHH is in solution with no other protein added, use the kit size that spans the amount of VHH you wish to label. For labeling 20 ug, we recommend using the 5-20 ug kit.
    - If the VHH buffer contains other thiol-containing molecules or other proteins, we recommend purifying the VHH to remove them.
    - The kit is compatible with VHH in ≤10% glycerol.
    - Buffers such as HEPES, MES, Tris, or phosphate, as well as additives like TCEP, EDTA, and sodium azide, are compatible with the reaction.
    - The kits are optimized for labeling VHH amounts at the middle point of the kit range (i.e., 12.5 ug for the 5-20 ug kit, or 35 ug for the 20-50 ug kit).
- The optimal protein concentration for the reaction is 1 mg/mL. If it is not feasible to obtain ≥1 mg/mL of the protein, we recommend proceeding with a concentration as close to 1 mg/mL as possible.

Standard Labeling Protocol

1. Warm up the Mix-n-Stain™ Reaction Buffer, Activating Agent, and Quenching Buffer vials to room temperature. Add 15 uL of water to the Activating Agent vial to prepare a 10X solution. Vortex each vial to mix before use. Centrifuge the vials briefly to collect the solutions at the bottom of the vials.
2. Start with your stock VHH concentration ≥1 mg/mL and transfer an appropriate ug amount of VHH (within the range of your kit) to a clean tube. For example, if your VHH is at 1 mg/mL, for a 10 ug-scale labeling reaction, use 10 uL of VHH. Calculate the correct volume to use based on your desired labeling scale and the concentration of your VHH.
3. Add the 5X Mix-n-Stain™ Reaction Buffer to the VHH solution at a ratio of 1:5 such that the solution contains a final concentration of 1X Reaction Buffer. For example, for every 10 uL of VHH solution, add 2.5 uL of 5X Reaction Buffer. Mix completely by pipetting up and down or gentle vortexing.
4. Add 10X Activating Agent to the mixture at a ratio of 1:10 such that the solution contains a final concentration of 1X Activating Agent. Add 1 uL for every 9 uL of VHH + Reaction Buffer from step 3. For example, for 10 uL VHH + 2.5 uL Reaction Buffer, add 1.4 uL Activating Agent. Mix thoroughly by pipetting up and down or gentle vortexing.
5. Centrifuge the vial briefly to collect the solution at the bottom of the vial.
6. Transfer the entire mixture from step 5 to the Dye Vial containing lyophilized dye. Resuspend the dye by pipetting up and down or gentle vortexing.
7. Incubate the Dye Vial containing the reaction in the dark for 2 hours at room temperature. Incubating for longer times will not affect the labeling.
8. Add the 10X Mix-n-Stain™ Quenching Buffer to the reaction vial at a ratio of 1:10 (1 uL of Quenching Buffer for every 9 uL of solution from step 4 and mix). For example, if you started with 10 uL VHH in a total volume of 13.9 uL after adding other reaction components, you would add 1.5 uL of Quenching Buffer.
9. Incubate the reaction vial for 10 minutes in the dark at room temperature. The VHH is now ready for use for staining. The concentration of the conjugate is the starting ug amount of VHH divided by the total volume.

Storage of Labeled VHH

We recommend adding 4X Mix-n-Stain™ Storage Solution at a ratio of 1:4 such that the solution contains a final concentration of 1X Storage Solution (1 uL of 10X Mix-n-Stain™ Storage Solution for every 3 uL of conjugate solution) to the labeled conjugate for storage at 4°C. For example, if you started with 10 uL VHH in a total volume of 15.4 uL, you would add 5.2 uL of Storage Solution. The final concentration of conjugate (10 ug in 20.6 uL final volume) would be 0.485 mg/mL.

Alternatively, conjugates can be stored in single use aliquots at -20°C without adding Storage Solution. 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 VHH for multi-color staining, or will the dye transfer between proteins?	Mix-n-Stain™ labeling results in covalent linkage of dye and VHH. 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 VHH?	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 VHH and Mix-n-Stain™ Nanobody Thiol Labeling Kits are optimized for labeling VHH bearing a single cysteine (1x Cys). 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 Antibody or Protein Labeling Kits).
If my VHH 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 VHH 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 one labeling reaction. We do not recommend that you try to split the kit to label for more than one reaction.
How important is the VHH concentration in the labeling reaction?	The kits are optimized for labeling VHH 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.

Related Products

Cat. No.	Product
20882-20887	Goat Anti-Alpaca IgG, VHH Antibody
23007, 23011	TrueBlack® Lipofuscin Autofluorescence Quencher
23014	TrueBlack® Plus Lipofuscin Autofluorescence Quencher, 40X in DMSO
23012	TrueBlack® IF Background Suppressor System (Permeabilizing)
33000-33020	Tyramide Amplification Kits
92170... 96128	CF® Dye Tyramide
30071	AccuOrange™ Protein Quantitation Kit
22030	AntiFix™ Universal Antigen Retrieval Buffer, 10X
41033... 41040	NucSpot® Nuclear Stains
40061	RedDot™2 Far-Red Nuclear Stain, 200X in DMSO
40009... 40043	DAPI
23001, 23002	EverBrite™ Mounting Medium (with or without DAPI)
23003... 23016	EverBrite™ Hardset Mounting Medium (with or without DAPI or NucSpot® 640)
23017-23019	EverBrite TrueBlack® Hardset Mounting Medium (with or without DAPI or NucSpot® 640)
23008, 23009	Drop-n-Stain EverBrite™ Mounting Medium (with or without DAPI)
23005	CoverGrip™ Coverslip Sealant
23023, 23024	Super^{HT} PAP Pen 2.0
23006	Flow Cytometry Fixation/Permeabilization Kit
22023	Paraformaldehyde, 4% in PBS, Ready-to-Use Fixative
22015	Fixation Buffer
22016	Permeabilization Buffer
22017	Permeabilization and Blocking Buffer (5X)
22013	Bovine Serum Albumin Fraction V
22014	Bovine Serum Albumin 30% Solution
22010	10X Fish Gelatin Blocking Agent
22011	Fish Gelatin Powder

Other Antibody or Protein Labeling Kits

Cat. No.	Product
92500-92515	Mix-n-Stain™ Nanobody Labeling Kits
92230... 92584	Mix-n-Stain™ CF® Dye Antibody Labeling Kits
92558-92575	Mix-n-Stain™ CF® Dye IgM Antibody Labeling Kits
92549... 92557	Mix-n-Stain™ STORM CF® Dye Antibody Labeling Kits
92294-92296	Mix-n-Stain™ FITC Antibody Labeling Kits
92412... 92418	Mix-n-Stain™ Cyanine Dye Antibody Labeling Kits
92244... 92444	Mix-n-Stain™ Biotin Antibody Labeling Kits
92328... 92450	Mix-n-Stain™ Digoxigenin Antibody Labeling Kits
92325-92327	Mix-n-Stain™ DNP Antibody Labeling Kits
92404... 92454	Mix-n-Stain™ Maxi Antibody Labeling Kits
92350... 92364	Mix-n-Stain™ Small Ligand Labeling Kits
92160-92163	VivoBrite™ Rapid Antibody Labeling Kits for Small Animal In Vivo Imaging
92208... 92228	CF® Dye & Biotin SE Protein Labeling Kits

Please visit our website at www.biotium.com to view our full selection of fluorescent CF® Dye antibody conjugates and reactive dyes, fluorescent probes, cellular stains, and reagents for immunofluorescence microscopy and flow cytometry.