

# Product Information

## VersaBlot™ Total Protein Normalization Kits

### Catalog Number

33025, 33025-T VersaBlot™ CF@680T Total Protein Normalization Kit  
33026, 33026-T VersaBlot™ CF@770T Total Protein Normalization Kit

### Size

33025, 33026 1000 labeling reactions or 50 mini gels per kit  
33025-T, 33026-T 200 labeling reactions or 10 mini gels per kit

### Storage and Handling

Store the Reactive CF@ Dyes at -20°C, protect from light.  
For convenience, all other components can be stored at 4°C. Protect the Reversal Buffer from light.  
Stable for at least 6 months from date of receipt when stored as recommended.

### Components

Component	33025	33025-T	33026	33026-T
Reactive CF@680T (92400A)	5 x 1 vial	1 vial	NA	NA
Reactive CF@770T (92401A)	NA	NA	5 x 1 vial	1 vial
WB Prestaining Buffer (99841)	2 x 1 mL	1 mL	2 x 1 mL	1 mL
High Sensitivity Prestaining Buffer (99842)	2 x 1 mL	1 mL	2 x 1 mL	1 mL
Reversal Buffer (99852)	500 mL	100 mL	500 mL	100 mL
Dye Reversal Agent (99853)	500 uL	100 uL	500 uL	100 uL
DMSO, Anhydrous (99953)	150 uL	150 uL	150 uL	150 uL

### Product Description

VersaBlot™ Total Protein Normalization Kits are designed for rapid and sensitive protein detection on SDS-PAGE gels and western blot (WB) membranes. The proteins are covalently labeled with near-infrared CF@ Dyes so that after electrophoresis, the bands can be directly visualized via fluorescent gel scanning, eliminating the need for any gel staining procedures. The labeled proteins on SDS-PAGE gels can then be transferred to membranes for WB normalization. If desired, the pre-stain can be reversed after scanning, facilitating downstream multi-color WB using antibodies conjugated to near-infrared CF@ Dyes in the same channel.

To label a protein or cell lysate sample, one simply needs to mix the dye and buffer with the protein solution, followed by a brief incubation at room temperature. The sample is then ready for denaturation and gel electrophoresis. No purification is needed. The excess CF@ Dye runs to the lowest end of gels, and does not interfere with visualization of the protein bands (see Figure 1A). The total protein staining can be detected either on the gel or on the membrane. After detection, reversal solution can be used to remove the staining from blotting membranes for subsequent multiplex fluorescent WB (see Figure 1).

For SDS-PAGE gel prestaining, the kits allow detection of low concentration proteins down to 1 ng (see Figure 2) with minimal background. The dyes do not cause any visible change to the shape or mobility of the bands compared to unlabeled proteins visualized by post-staining methods.

For WB normalization, the kits demonstrate outstanding linearity for quantification of total protein contents over a wide dynamic range (0.1 - 15 ug), outperforming the traditional normalization method based on housekeeping proteins (see Figure 1D). Compared to total protein post-staining products like the Ponceau S and LI-COR® REVERT™ Total Protein Stain, VersaBlot™ features a prestaining protocol with superior signal-to-noise and sensitivity (able to detect 10% difference in protein content), with comparable reversibility and ease-of-use. The labeling and reversal procedures do not affect protein mobility or detection by antibodies (including phosphoprotein and glycoprotein detection).

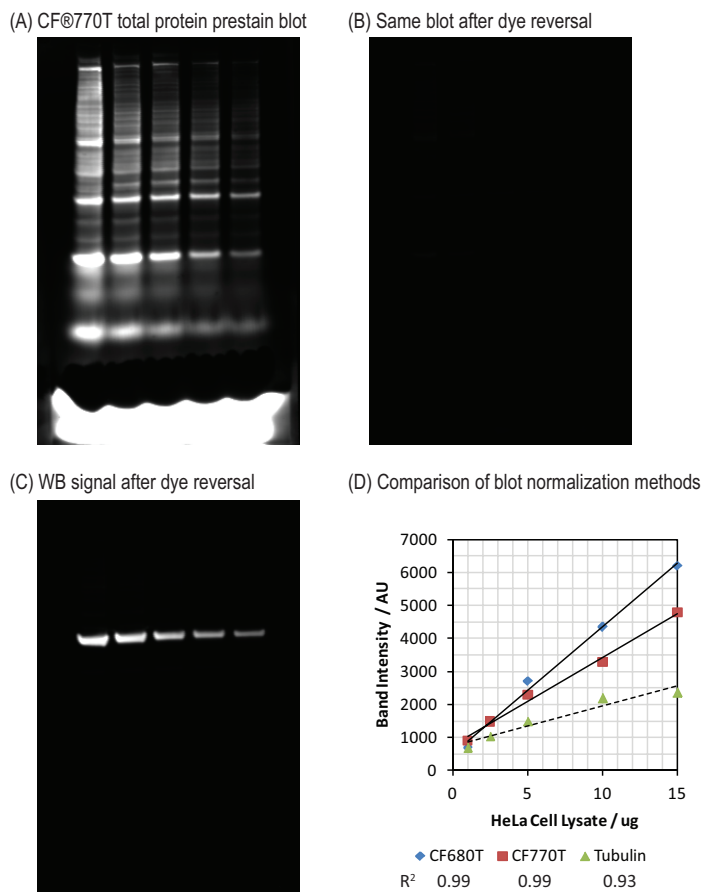


Figure 1. VersaBlot™ Total Protein Normalization Kits for WB normalization. (A) HeLa cell lysates in serial dilution were labeled with the CF@770T kit and visualized on a PVDF membrane. (B) On the same membrane, the fluorescence was efficiently removed after reversal. (C) The same membrane was then used for WB detection of tubulin using a CF@770 conjugated secondary antibody. The total protein staining and reversal does not affect sensitivity or background of the WB. (D) Plots of band intensity vs. protein content for CF@680T and CF@770T labeled lysate compared to tubulin WB. The VersaBlot™ Total Protein Normalization Kits showed better linearity compared to the antibody labeling of housekeeping proteins.

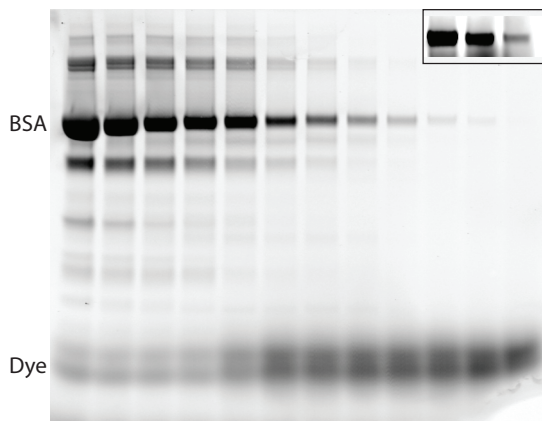
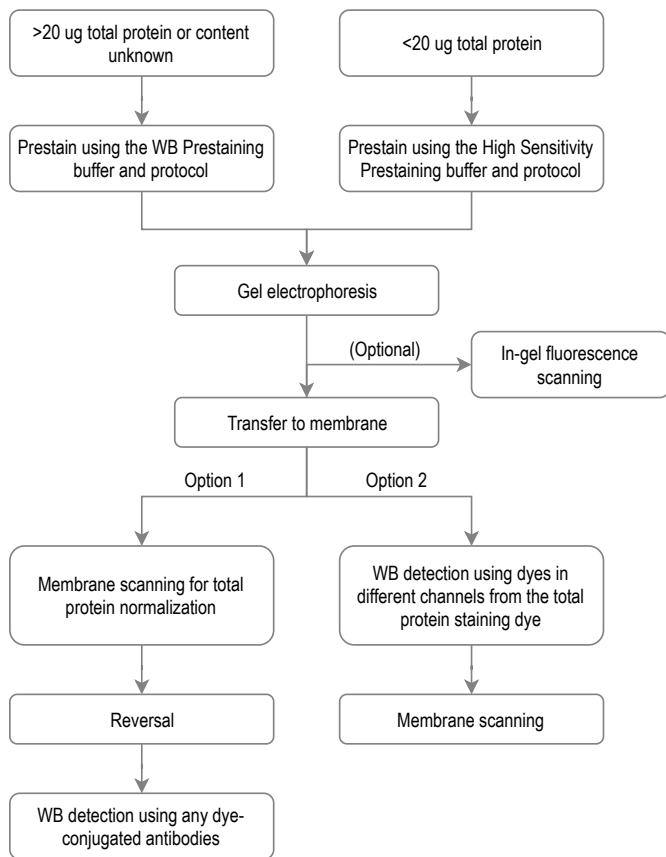


Figure 2. In-gel fluorescence image of bovine serum albumin (BSA) labeled with the VersaBlot™ CF@680T Total Protein Normalization Kit on SDS-PAGE gel. Protein content for each lane ranges from 10 ug to 1 ng, from left to right. The bands above and below the major bands are from impurity proteins in the BSA sample. The excess dye runs to the very bottom of the gel. Inset: part of the image with enhanced brightness to visualize the bands with 10, 5, and 1 ng of BSA.

### Protocol Overview



### Protein Sample Compatibility

The kits have been proven to work robustly with protein samples in various buffer systems, including water, PBS, Tris and HEPES buffers, with or without common detergents (Tween®-20, Triton™ X-100, NP-40). The kits tolerate most salts and compounds that are commonly present in protein samples, such as NaCl, KCl, sucrose, EDTA, protease inhibitor cocktails, and cell culture media.

The kits tolerate SDS up to 2% and glycerol up to 10%; therefore they can be used for protein samples in SDS-PAGE loading buffers, labeling both native and denatured proteins. The tracking dyes in SDS-PAGE loading buffers do not interfere with the near-infrared CF@ Dyes in the kits.

The kits do not efficiently label proteins in presence of high concentration (>10 mM) dithiothreitol (DTT). Instead, DTT can be added after the prestaining, which does not affect the labeled proteins.

### Choosing the Right Dye

The VersaBlot™ Total Protein Normalization Kits enable multi-color protein detection on membranes, with or without reversal. It is important to maintain balanced signal intensity across all channels for multi-color imaging (i.e. not having one color dramatically brighter than others). If signal crosstalk is a concern, we recommend to use the VersaBlot™ CF@770T Total Protein Normalization Kit.

The spectral properties of the near-infrared CF@ Dyes and recommended instrument settings on popular gel imaging systems are listed in the following table.

Table 1. Detection settings for CF@680T and CF@770T

Dye	Abs / Em	Imaging System	Excitation	Emission Filter
CF@680T	681 nm / 698 nm	Amersham Typhoon™ Trio; Amersham Typhoon™ RGB	630 nm	670BP30
		Amersham Typhoon™ 5; Amersham Typhoon™ NIR	685 nm	720BP20
		Amersham Imager 680 RGB	630 nm	705BP40
		UVP ChemStudio and UVP ChemStudio PLUS	660 nm (IR1)	730 nm
		LI-COR® Odyssey®; Odyssey® CLx	700 channel	
		ChemiDoc™ MP Imaging System (Bio-Rad)	Far-red channel	
CF@770T	764 nm / 787 nm	Amersham Typhoon™ 5; Amersham Typhoon™ NIR	785 nm	825BP30
		UVP ChemStudio and UVP ChemStudio PLUS	785 nm (IR2)	810 nm
		LI-COR® Odyssey®; Odyssey® CLx	800 channel	
		ChemiDoc™ MP Imaging System (Bio-Rad)	NIR channel	
		Azure C500; Azure C600, Azure Sapphire Imager	785 channel	

### Choosing the Right Buffer

Two different buffers are provided in each kit: the WB Prestaining Buffer and the High Sensitivity Prestaining Buffer. Choose one buffer with the corresponding prestaining protocol for each sample based on your applications.

Use the WB Prestaining Buffer and protocol for samples with > 20 ng/uL of total proteins. This protocol is optimized for WB total protein content normalization. The signal is linear for total protein content of 0.1 - 15 ug in 10 uL of sample. For in-gel fluorescence detection the linearity range is 0.2 - 40 ug.

Use the High Sensitivity Prestaining Buffer and protocol for samples with < 20 ng/uL of total proteins. This buffer is optimized for detection of low concentrations of protein with minimal background. The signal is linear for total protein content of 1 - 200 ng in 10 uL of sample.

If the total protein concentration is unknown, we recommend performing a preliminary test labeling with the WB Prestaining Buffer. If the protein bands are dim or the background is high, switch to the high sensitivity buffer. For native protein gel electrophoresis we recommend to use the high sensitivity buffer.

### WB Prestaining Protocol (total protein > 20 ng/uL)

This protocol is optimized for total protein content normalization for western blotting.

1. Warm up the CF® Dye and the WB Prestaining Buffer to room temperature. It is normal to see white precipitates in the WB Prestaining Buffer. A gentle vortex at room temperature or briefly warming at 37°C will help to dissolve the solids. Centrifuge the vials briefly to collect the solutions at the bottom of the vials.
2. Add 20 uL of DMSO to one vial of CF® Dye to make the CF® Dye stock solution. The CF® Dye stock solution can be stored at -20°C for 6 months.
3. Prepare the working solution by diluting the CF® Dye stock solution 10-fold in WB Prestaining Buffer. For example, mix 1 uL CF® Dye stock solution with 9 uL WB Prestaining Buffer (see Note 1). This working solution can be used to label 10 X 10 uL samples (or a total of 100 uL of your protein sample).
4. Add 1/10 volume of the working solution to the protein sample. For example, if you have 10 uL of protein sample, add 1 uL of working solution. Mix well by gently vortexing the sample vial.
5. Incubate the sample at room temperature for 30 minutes. Protect from light.
6. The sample is now ready for denaturation and SDS-PAGE. Treat the sample with SDS-PAGE loading buffer and reducing reagent (if necessary) according to your standard protocol and then load on gels. If labeling a denatured protein sample that is already in SDS-PAGE loading buffer, it can be directly loaded on gels after prestaining.
7. After SDS-PAGE, the protein bands can be visualized on the gel using a gel imaging system. See Table 1 for suggested instrument settings on popular gel imaging systems. If performing western blotting, continue to the Blotting and Dye Reversal Protocol.

### High Sensitivity Prestaining Protocol (total protein < 20 ng/uL)

This protocol is optimized for detection of low concentrations of protein.

1. Warm up the CF® Dye and the High Sensitivity Buffer to room temperature. Centrifuge the vials briefly to collect the solutions at the bottom of the vials.
2. Add 20 uL of DMSO to one vial of CF® Dye to make the CF® Dye stock solution. The CF® Dye stock solution can be stored at -20°C for 6 months.
3. Prepare the working solution by mixing 1 uL of CF® Dye stock solution with 99 uL of the High Sensitivity prestaining buffer. This working solution can be used to label a total of 1000 uL of protein samples. The working solution should be used within 1 hour after preparation.
4. Add 1/10 volume of the working solution to the protein sample. For example, if you have 10 uL of protein sample, add 1 uL of the working solution. Mix well by gently vortexing the sample vial.
5. Incubate the sample at room temperature for 30 minutes. Protect from light.
6. The sample is now ready for gel electrophoresis. For SDS-PAGE, treat the sample with SDS-PAGE loading buffer and reducing reagent (if necessary) according to your standard protocol and then load gel.
7. After SDS-PAGE, the protein bands can be visualized on the gel using a gel imaging system. See Table 1 for suggested instrument setting on popular gel imaging systems. If performing western blotting, continue to the Blotting and Dye Reversal Protocol.

### Blotting and Dye Reversal Protocol

1. For WB normalization, transfer the proteins to a membrane according to your standard protocol. The protein bands can be detected by scanning the membrane on a gel imaging system, either before or after incubating with antibodies to detect specific proteins (see Note 2). Membrane blocking, antibody binding, washing and stripping does not affect the fluorescence signal. The excess CF® Dye on the lowest end of the membrane will be washed off during WB process.
2. To reverse the fluorescence of CF® Dye total protein staining, dilute the Reversal Agent 1:1000 in the Reversal Buffer and incubate the membrane in this reversal solution. For example, add 10 uL of the Reversal Agent to 10 mL of Reversal Buffer to cover a 7 cm X 8.4 cm membrane in a 9 cm X 9 cm dish. Incubate at room temperature for 30 minutes on an orbital shaker. The membrane is now ready for multi-color WB detection (see Note 3).

Note 1: If the total protein stain gives very strong fluorescence that saturates the imaging device, use less CF® Dye to make the working solution. We have achieved bright staining using 10% of the recommended amount of CF® Dye.

Note 2: For general procedures and tips for fluorescent western blotting, download the Protocols for Antibody Based Detection on our product page.

Note 3: We found that this condition removes >99% of the fluorescence of total protein staining, which is sufficient for most WB applications. If higher reversal efficiency is desired, repeat step 3 two to three times, by decanting the used reversal solution and adding new reagent for each reversal step.

### Related products

Catalog #	Product Name
21003	One-Step Blue® Protein Gel Stain
21004	One-Step Lumitein™ Protein Gel Stain
21005	One-Step Lumitein™ UV Protein Gel Stain
20065	CF®680 Goat Anti-Mouse IgG (H+L), Highly Cross-Adsorbed
20067	CF®680 Goat Anti-Rabbit IgG (H+L), Highly Cross-Adsorbed
20192	CF®680R Goat Anti-Mouse IgG (H+L), Highly Cross-Adsorbed
20193	CF®680R Goat Anti-Rabbit IgG (H+L), Highly Cross-Adsorbed
22077	CF®770 Goat Anti-Mouse IgG (H+L), Highly Cross-Adsorbed
20078	CF®770 Goat Anti-Rabbit IgG (H+L), Highly Cross-Adsorbed
22010	10X Fish Gelatin Blocking Agent
90082	DMSO, anhydrous
22020	10X Phosphate Buffered Saline (PBS)
22014	Bovine Serum Albumin 30% Solution
22001	Ponceau S solution
21530	Peacock™ Prestained Protein Marker
21531	Peacock™ Plus Prestained Protein Marker
23013	TrueBlack® WB Blocking Buffer Kit

Please visit [www.biotium.com](http://www.biotium.com) to view our full selection of products featuring bright and photostable CF® dyes, including primary antibodies, secondary antibodies, streptavidin, anti-biotin, and anti-tag antibodies. Biotium also offers a variety of products for antibody labeling and protein gel staining.

CF is a registered trademark of Biotium, Inc. CF dye, Mix-n-Stain, and modified Mix-n-Stain labeling technologies are covered by pending US and international patents. Coomassie is a trademark of Imperial Chemical Industries; LI-COR and Odyssey are registered trademarks of LI-COR Biosciences; REVERT is a trademark of LI-COR Biosciences; Typhoon is a trademark of GE Healthcare; TWEEN is a registered trademark of Croda International PLC; Triton is a trademark of Dow Chemical Company.

Biotium products are high-quality reagents and materials intended for research purposes only. Some products are potentially hazardous chemicals - please read the Material Safety Data Sheet for additional information regarding handling potentially hazardous chemicals. Several of Biotium products and product applications are covered by U.S. and international patents and pending patents. Our products are not available for resale or other commercial uses without a specific agreement from Biotium, Inc. We welcome inquiries about licensing the use of our dyes, trademarks or technologies. Please submit inquiries by e-mail to [btinfo@biotium.com](mailto:btinfo@biotium.com).