# Product Information

## VersaBlot™ Total Protein Normalization Kits

### Kit Contents

| Component | 33025-T 200 x 10 uL reactions | 33025 1000 x 10 uL reactions | 33026-T 200 x 10 uL reactions | 33026 1000 x 10 uL reactions |
|---|---|---|---|---|
| Reactive CF®680T (92400A) | 1 x 1 vial | 5 x 1 vial | NA | NA |
| Reactive CF®770T (92401A) | NA | NA | 1 x 1 vial | 5 x 1 vial |
| WB Prestaining Buffer (99841) | 1 x 1 mL | 2 x 1 mL | 1 x 1 mL | 2 x 1 mL |
| High Sensitivity Prestaining Buffer (99842) | 1x 1 mL | 2 x 1 mL | 1 x 1 mL | 2 x 1 mL |
| Reversal Buffer (99852) | 100 mL | 500 mL | 100 mL | 500 mL |
| Dye Reversal Agent (99853) | 100 uL | 500 uL | 100 uL | 500 uL |
| DMSO, Anhydrous (99953) | 150 uL | 150 uL | 150 uL | 150 uL |

### Storage and Handling

Store dye component at -20°C, protect from light. For convenience, all other components can be stored at 4°C. Protect the Reversal Buffer from light. Product is stable for at least 6 months from date of receipt when stored as recommended.

CF® dye stock solutions prepared in anhydrous DMSO can be aliquoted and stored with desiccant and protected from light at -20°C, for up to 6 months.

### 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 fluorescence 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 prestain 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 bottom of the gels, and does not interfere with visualization of the protein bands (Figure 2A). The total protein staining can be detected either in the gel or after transfered to membrane. After detection, reversal solution can be used to remove the staining from blotting membranes for subsequent multiplex fluorescent WB (Figure 2).

For SDS-PAGE gel prestaining, the kits allow detection of low concentration proteins down to 1 ng (Figure 1) 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 content over a wide dynamic range (0.1 - 15 ug), outperforming the traditional normalization method based on housekeeping proteins (Figure 2D). Compared to other reversible total protein staining methods like Ponceau S and LI-COR® REVERT™ Total Protein Stain, VersaBlot™ features superior signal-to-noise and sensitivity, allowing detection of as little as 10% difference in protein content between samples. The labeling and reversal procedures do not affect protein mobility or detection by antibodies, including phosphoprotein and glycoprotein detection.

### Experimental Protocols

#### Protein Sample Compatibility

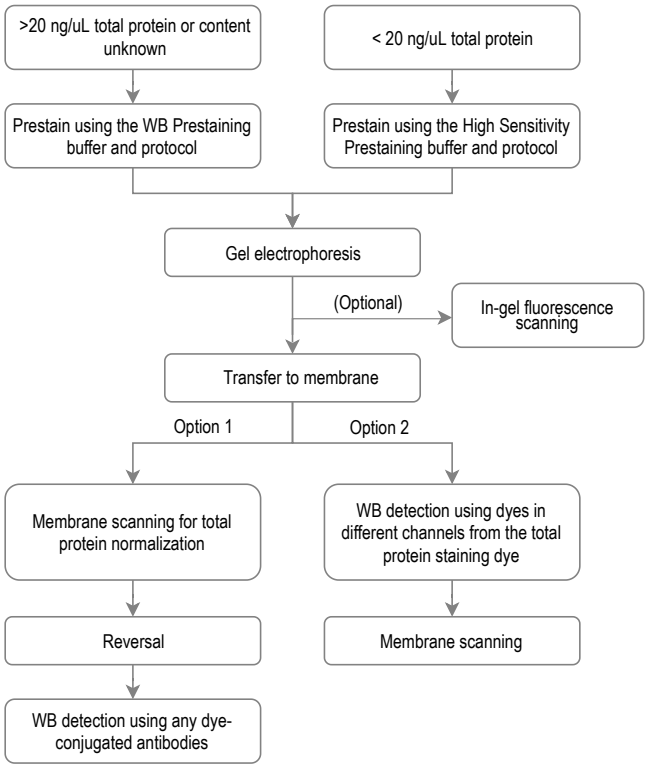
The kits have been proven to work robustly with protein samples in various buffer systems, including water, PBS, Tris and HEPES buffers, with or without common detergents (Tween®-20, Triton® X-100, NP-40). The kits tolerate most salts and compounds that are commonly present in protein samples, such as 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 the presence of high concentrations (>10 mM) of reducing agents like dithiothreitol (DTT) or beta-mercaptoethanol (BME). If required, add reducing agents after the prestaining reaction is complete.

#### 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 Prestaining Buffer. For native protein gel electrophoresis we recommend to use the High Sensitivity Buffer.

### 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 | |
| | | Azure C500; Azure C600, Azure Sapphire Imager | 660 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 | |

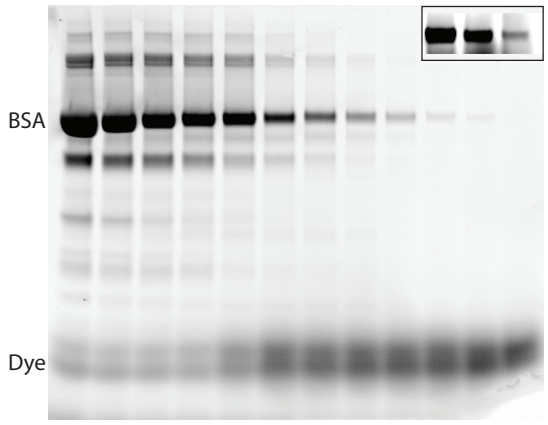



Figure 1. In-gel fluorescence image of bovine serum albumin (BSA) labeled with the VersaBlot™ CF®680T Total Protein Normalization Kit on SDS-PAGE gel. Protein content for each lane ranges from 10 ug to 1 ng, from left to right. The bands above and below the major bands are from impurity proteins in the 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.

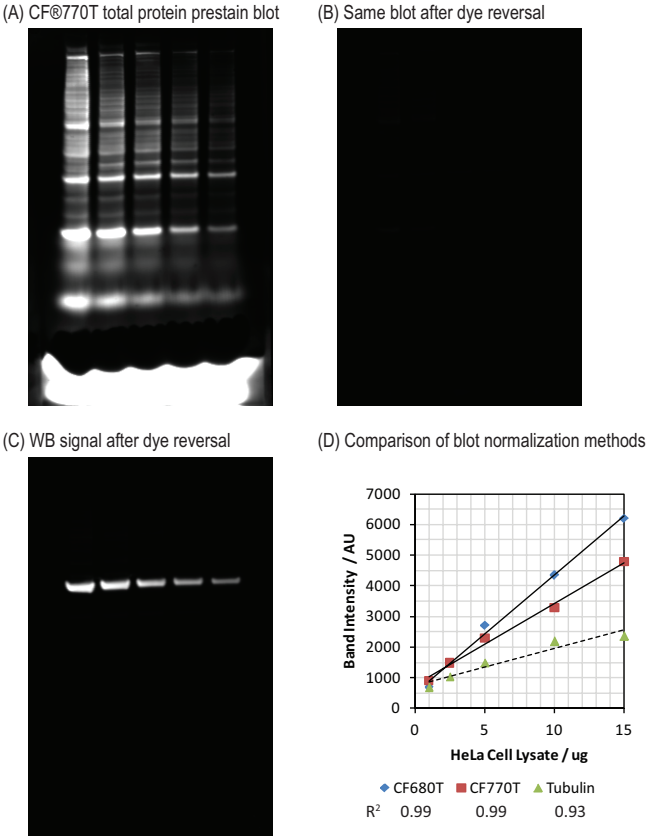



Figure 2. VersaBlot™ Total Protein Normalization Kits for WB normalization. (A) HeLa cell lysates in serial dilution, 15 - 1 ug/lane, were labeled with the CF®770T kit and visualized on a PVDF membrane. (B) On the same membrane, the fluorescence was efficiently removed after reversal. (C) The same membrane 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. VersaBlot™ Total Protein Normalization Kits showed better linearity compared to antibody labeling of housekeeping proteins.

**1. WB Prestaining Protocol (total protein >20 ng/uL)**

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

1.1 Warm up CF® dye and WB Prestaining Buffer to room temperature. It is normal to see white precipitates in the WB Prestaining Buffer. Gently vortex at room temperature or briefly warm to 37°C to help redissolve the solids. Centrifuge the vials briefly to collect the solutions at the bottom of the vials.

1.2 Add 20 uL DMSO to one vial of CF® dye to make CF® dye stock solution.

1.3 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. Working solution can be used to label 10 x 10 uL samples (or a total of 100 uL of your protein sample).

1.4 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.

**Note:** If the total protein stain gives 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.

1.5 Incubate the sample at room temperature for 30 minutes. Protect from light.

1.6 If the sample is in SDS-PAGE loading buffer, it can be directly loaded onto the gel. If necessary, treat the sample with SDS-PAGE loading buffer and reducing reagent according to your standard protocol and then load the gel.

1.7 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.

**2. High Sensitivity Prestaining Protocol (total protein <20 ng/uL)**
This protocol is optimized for detection of low concentrations of protein.

2.1 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.

2.2 Add 20 uL of DMSO to one vial of CF® dye to make CF® dye stock solution.

2.3 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.

2.4 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.

2.5 Incubate the sample at room temperature for 30 minutes. Protect from light.

2.6 If the sample is in SDS-PAGE loading buffer, it can be directly loaded onto the gel. If necessary, treat the sample with SDS-PAGE loading buffer and reducing reagent according to your standard protocol and then load the gel.

2.7 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.

**3. Blotting and Dye Reversal Protocol**

3.1 For WB normalization, transfer the proteins to a membrane according to your standard protocol.

**Note:** 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. 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 the WB process.

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

3.2 To reverse the fluorescence of CF® dye total protein staining, dilute the Reversal Agent 1:1000 in the Reversal Buffer. 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.

3.3 Incubate the membrane in reversal solution at room temperature for 30 minutes on an orbital shaker. The membrane is now ready for multi-color WB detection.

**Note:** We found that this protocol removes >99% of the fluorescence of total protein staining, which is sufficient for most WB applications. For higher reversal efficiency, repeat steps 3.2 & 3.3 two to three times, by decanting the used reversal solution and adding new reversal solution.

**Related Products**

| Catalog number | Product |
|---|---|
| 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 |
| 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 |
| 30071 | AccuOrange™ Protein Quantitation Kit |
| 33021 | GloMelt™ Thermal Shift Protein Stability Kit without ROX |
| 33022 | GloMelt™ Thermal Shift Protein Stability Kit with ROX |

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