Product Information

Revised: September 10, 2018

Mix-n-Stain™ Total Protein Prestain Kits

Catalog number:

92400, 92400-T Mix-n-Stain™ CF®680T Total Protein Prestain Kits

92401, 92401-T Mix-n-Stain™ CF®770T Total Protein Prestain Kits

Size:

92400, 92401 1000 labeling reactions per kit

92400-T, 92401-T 200 labeling reactions per kit

Storage: -20°C. Protect from light.

Stability: Stable for at least 12 months from date of receipt when stored as recommended.

Components:

Component	92400 (1000 labelings)	92400-T (200 labelings)	92401 (1000 labelings)	92401-T (200 labelings)
Reactive CF®680T (92400A)	5 x 1 vial	1 x 1 vial	NA	NA
Reactive CF®770T (92401A)	NA	NA	5 x 1 vial	1 x 1 vial
WB Prestaining Buffer (99841)	2 x 1 mL	1 x 1 mL	2 x 1 mL	1 x 1 mL
High Sensitivity Prestaining Buffer (99842)	2 x 1 mL	1 x 1 mL	2 x 1 mL	1 x 1 mL
DMSO, Anhydrous (99953)	1 x 150 uL	1 x 150 uL	1 x 150 uL	1 x 150 uL

Product Description

Mix-n-Stain™ Total Protein Prestain 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 and detected by WB.

To label a protein sample, one simply needs to mix the dye and buffer with the protein solution, followed by a brief incubation at room temperature. The protein sample is then ready for denaturation and gel electrophoresis. No purification is needed. The excess CF® dye runs to the lowest end of gel, and does not interfere with visualization of the protein bands. If used for WB, the excess dye will be washed off the membrane, leaving only the labeled proteins.

For SDS-PAGE gel prestaining, the kits allows detection of low concentration proteins down to 1 ng (see 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 applications, the kits demonstrate outstanding linearity for quantification of total protein contents over a wide dynamic range, outperforming the traditional normalization method based on housekeeping proteins (see Figure 2). The prestaining does not affect protein detection by antibodies.

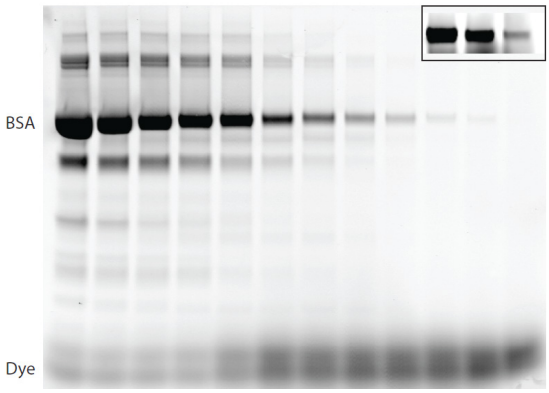

Figure 1. In-gel fluorescence image of bovine serum albumin (BSA) labeled with the CF®680T Total Protein Prestain Kit on SDS-PAGE gel. Protein content for each lane ranges from 10 ug to 1 ng, from left to right. The bands above and below the major bands are from impurity proteins in the BSA sample. The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

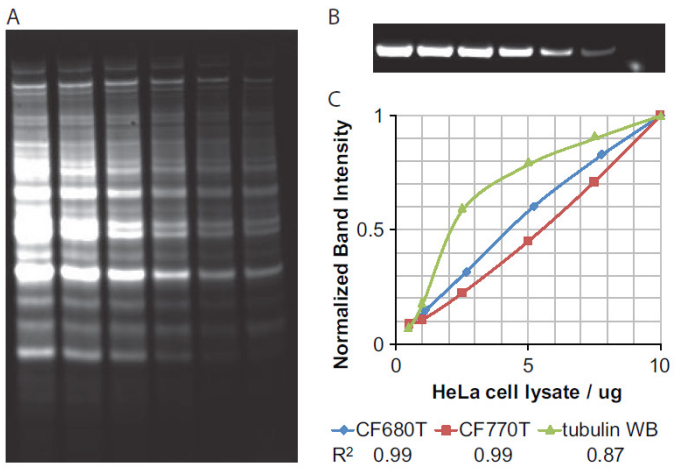

Figure 2. Mix-n-Stain™ Total Protein Prestain Kits for WB normalization. HeLa cell lysates in serial dilution are labeled with (A) the CF®770T Total Protein Prestain Kit or (B) mouse anti-tubulin primary antibody and CF®770T goat anti-mouse secondary antibody, both on PVDF membranes. Total protein content in cell lysate was determined by the bicinchoninic acid assay. (C) Plots of band intensity vs protein content for CF®770T labeled lysate (shown in A), CF®680T labeled lysate (membrane not shown), and tubulin WB (shown in B). The Mix-n-Stain™ Total Protein Prestain Kits show better linearity compared to the antibody labeling of housekeeping proteins.

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 can 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

Unlike Coomassie™ Brilliant Blue or other post-staining dyes with broad absorption and emission spectra, the bright near-infrared CF® dyes in the kits can be optically separated with a large variety of fluorescently labeled antibodies, allowing multi-color detection. 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 suggest to use the CF®770T total protein staining kit to compliment WB detection using our CF®680 or CF®680R conjugated secondary antibodies.

The spectral properties of the near-infrared CF® dyes and recommended instrument settings on popular gel imaging systems are listed in the following table:

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
		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
		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 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 0.2 - 40 ug in 10 uL of sample.

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 mixing 1 uL of CF® dye stock solution with 9 uL of the WB prestaining buffer. This working solution can be used to label a total of 100 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 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.
7. After SDS-PAGE, the gel can be scanned using an imaging system to visualize the proteins bands, otherwise the proteins can be transferred to a membrane by western blotting.
8. For western blot 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. Membrane blocking, antibody binding, washing and stripping does not affect the fluorescence signal.

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

Optimized for detection of low concentrations of protein.

1. Warm up the CF® dye and the WB 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 electrophoresis, use an imaging system (see table) to detect proteins in the gel, or to detect proteins on a membrane after western blot transfer.

Related products

Catalog #	Product Name	Unit Size
21003	One-Step Blue® Protein Gel Stain	1 L
21004	One-Step Lumitein™ Protein Gel Stain	1 L
21005	One-Step Lumitein™ UV Protein Gel Stain	1 L
22010	10X Fish Gelatin Blocking Agent	100 mL
90082	DMSO, anhydrous	10 mL
22020	10X Phosphate Buffered Saline (PBS)	4 L
22014	Bovine Serum Albumin 30% Solution	100 mL
22001	Ponceau S solution	1 L
21530	Peacock™ Prestained Protein Marker	50 uL or 500 uL
21531	Peacock™ Plus Prestained Protein Marker	50 uL or 500 uL

Please visit **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.