TotalStain Q – NC Fluorescent Total Protein Staining Kit

Rapid fluorescent stain for total protein normalization of Western blots on nitrocellulose

Short Protocol for Catalog Number

AC2227 TotalStain Q – NC Fluorescent Total Protein Staining Kit

Description

TotalStain Q – NC is a rapid fluorescent stain for quantification of total protein on nitrocellulose membranes after the protein transfer step. TotalStain Q allows for the subsequent immunodetection of targets using chemiluminescence and near-infrared (NIR) fluorescence at 700nm and 800nm.

Its high affinity for proteins allows for sensitive and quantitative detection of bands for total protein normalization (TPN) and verification of transfer efficiency. The excitation and emission properties make TotalStain Q compatible with digital imaging systems capable of Cy3 detection.

For accurate, quantitative Western blots, it is critical to perform normalization to account for variability in both sample loading and transfer efficiency. Currently, TPN is becoming the standard for normalization and the preferred method suggested in top tier journals.

azure biosystems

Kit Contents

- S1091 – NC Staining Solution
- S1072 – Washing Solution
- S1092 – Imaging Folders

Additional Materials Required

- 100% Ethanol (use only pure, 200-proof ethanol).
- Staining tray.
- Shaking or rocking platform.

Storage Information

The TotalStain – Q NC Fluorescent Total Protein Staining Kit is stable at room temperature (4°-25°C) for at least one year.

Warnings and Precautions

- The TotalStain – Q NC Fluorescent Total Protein Staining Kit is for research use only.
- The TotalStain – Q NC Fluorescent Total Protein Staining Kit is compatible with nitrocellulose membranes.
- If staining PVDF membranes use our alternative product TotalStain Q – PVDF (AC2225).
- Always wear gloves when handling membranes and reagents.
- Refer to MSDS for additional safety information.
- The product is guaranteed to be free of manufacturer defects, and to function as described when the provided protocol is followed by properly trained personnel.

Preparation of Solutions

NC Working Staining Solution: Before using the product for the first time, add 30mL of 100% Ethanol directly to the bottle of NC Staining Solution and mix well. Check the box on the bottle's label. This only needs to be done one time.

Working Washing Solution: Before using the product for the first time, add 135ml of 100% Ethanol directly to the bottle of Washing Solution and mix well. Check the box on the bottle's label. This only needs to be done one time.

Short Protocol

Note: *This protocol is for staining one full-size mini-blot. If using blots of differing sizes, adjust the volume of staining and washing solution appropriately.*

1. Following the protein transfer, immerse the blot in at least 10mL of water for 5 minutes with gentle agitation.
2. Decant water and dispense 10mL of NC Working Staining Solution. Incubate for 10 minutes with gentle agitation.
3. Decant the NC Working Staining Solution and wash the membrane three times for 3 minutes per wash with 10mL Working Washing Solution.
4. Immediately place the blot in an Imaging Folder and image the **wet** blot using a digital imaging system in either the Cy3 or Safe Dye channel.
5. Continue with the Blocking Step for immunodetection of targets: Rinse with water before blocking. Compatible with chemiluminescent and NIR fluorescent immunodetection.

Troubleshooting and FAQ

TotalStain Q – NC Fluorescent Total Protein Staining Kit, is a quick and easy method for staining immunoblotted proteins on nitrocellulose membranes. It allows for TPN and verification of transfer efficiency prior to immunodetection. Some common questions are addressed below.

Problem	Possible Solutions
High background	• Increase washing time.
No or low signal	• Ensure effective transfer: check electrical source. Verify transfer was performed in the correct direction. Optimize the transfer time and voltage conditions. Ensure good contact was established between membrane and gel. Make sure proper buffer was used. • Ensure that the Nitrocellulose membrane is fully hydrated before incubating with stain.
White spots within bands	• Improve transfer, make sure to remove any air bubbles between the gel and the membrane.
Uneven staining	• Check that the blot is completely immersed in the staining solution. • Ensure even agitation by incubating on orbital shaker. • Improve transfer, making sure that the membrane and the gel are assembled correctly and tightly in your transfer apparatus, and adequate pressure is applied.

For More Information

Visit **www.azurebiosystem.com**.