Ponceau Staining Protocol for eStain™ L1 Protein Staining System

Application Note

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Ponceau Staining Protocol for eStain™ L1 Protein Staining System

Background

eStain™ L1 Protein Staining System is a highly efficient protein PAGE gel staining system, which uses Coomassie Brilliant Blue and a patented protein staining technology developed by Genscript. eStain™ staining system integrates the traditional three steps of fixing-staining-destaining into one step and can stain and destain two protein PAGE gels or membranes simultaneously within 10 minutes. GenScript also offers the eStain™ L1 Protein Staining Kit (Coomassie Brilliant Blue), which can be directly ordered on GenScript website.

In addition to eStain™ L1 Protein Staining Kit, GenScript developed a Ponceau staining protocol as an alternative reagent option for customers to use on eStain system.

Ponceau Staining Solution can be used for the detection of proteins on both PVDF and nitrocellulose membranes provided by GenScript. On PVDF and nitrocellulose membranes, transferred protein can be detected in red protein bands with minimum or no background. Similar to the original Coomassie Brilliant Blue, eStain also offers superior sensitivity with Ponceau staining solution and can detect as low as 12.5 ng of protein.

Ponceau is a negative stain which binds to the positively charged amino groups of the protein. It also binds non-covalently to non-polar regions in the protein.

Solution Ingredients

- Ponceau staining solution at working concentration contains 0.02% Ponceau (w/v) in 1% acetic acid (v/v) in ddH_2O.
- De-staining solution is 10% acetic acid (v/v).
- Store at room temperature.

Procedure for Ponceau staining on PVDF/NC membrane in eStain™ staining system

- After regular electrophoresis for 30 minutes at 200V in Tris-MOPS-SDS Running buffer, the proteins in the gel were transferred to a PVDF or NC membrane using the standard 17-minute protein transfer method in the eBlot™ device
- Ponceau staining solution is connected to the blue inlet of eStain™ device.
- The NC/PVDF membrane is placed on the fabric side of the eStain cassette holder.
- A piece of eStain filter paper pre-soaked with distilled water is placed on top of the membrane and the cassette is closed.
- Insert the closed cassette into the eStain cassette channel to perform the standard 9-minute staining procedure.
- The eStain™ device automatically stops running once the staining is completed.
- Take out the NC/PVDF membrane and set dry.

Results

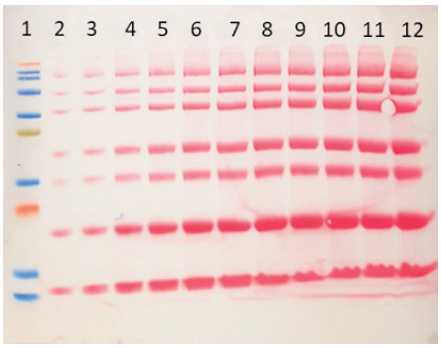

Figure 1. Ponceau staining on nitrocellulose membrane using eStain™ L1 Protein Staining System. Pre-stained protein Standard (cat# M006245) (Lane 1) and unstained PAGE-Master protein Standards (cat#M00516) were serially (2.5ul, 5ul, 10ul, 15ul, 20ul, 25ul, 30ul, 35ul, 40ul, 45ul, 50ul) (Lanes 2 to 12) loaded and run on 4-12% Bis-Tris Sure PAGE gel. The proteins in the gel were transferred to a NC membrane using the eBlot™ device. GenScript automated Ponceau stain was performed according to the protocol described above.

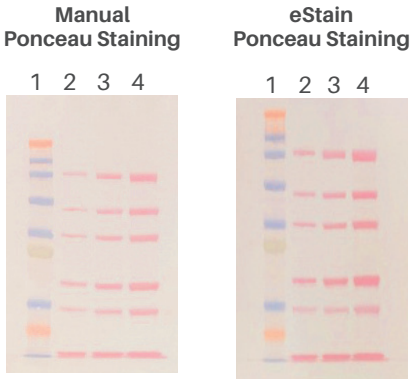

Figure 2. Ponceau staining on PVDF membrane manually (left) and using eStain™ L1 Protein Staining System (right). Pre-stained protein Standard (cat# M006245) was applied to gel (Lane 1) and unstained PAGE-Master protein Standard (cat#M00516) were serially loaded (2.5ul, 5ul, 10ul) (Lanes 2 to 4) and run on 4-12% Bis-Tris Sure PAGE gel. The proteins in the gel were transferred to a PVDF membrane using the eBlot™ device. On the left, a manual pounceau stain was preformed and, on the right, GenScript automated Pounceau stain was performed according to the protocol described above.

eStain™ L1

It takes several rounds of manual handling on solution exchange, membrane transfer and bench side waiting during at least 20-minute operating time. However, with eStain™ L1C staining system, the staining procedure with either Coomassie Brilliant Blue and Ponceau staining solution is fully automated and can be completed within 10 minutes, while providing outstanding staining quality. It is important for our customers to have consistent and high quality results during their gel experiments to avoid accidental waste of protein samples due to experimental error. Moreover, the remarkable homogeneous staining makes it suitable for protein quantification and normalization.

eStain™ L1 Staining System offers superior sensitivity and consistency with Ponceau staining solution on both NC and PVDF membranes. The bands are clear with no background and are comparable to the conventional manual staining method with much less labor. This alternative Ponceau staining protocol provides more flexibility in choosing the staining solution for eStain™ L1 Staining System.

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