VisULite MAX™ ECL Western Blotting Substrate

Catalog Number: VL002

Volume: 2 x 100 mL

PRODUCT DESCRIPTION

VisULite MAX™ ECL Western Blotting Substrate is a two component (Substrate A, Part # 898603 & Substrate B, Part # 898604) high sensitivity Enhanced Chemiluminescent (ECL) substrate. VisULite MAX™ ECL Western Blotting Substrate is compatible with PVDF and nitrocellulose membranes and various blotting buffers. Detection and analysis may be done using X-ray film or CCD imaging systems.

INTENDED USE

VisULite MAX™ ECL Western Blotting Substrate is for Western blots utilizing horseradish peroxidase (HRP)-conjugated detection components.

STABILITY & STORAGE

Upon receipt, VisULite MAX™ ECL Western Blotting Substrate can be stored in the dark at 2-8 °C for up to 12 months. Keep container closed tightly.

PRECAUTION

See MSDS.

LIMITATIONS

- FOR LABORATORY RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- This reagent should not be used beyond the expiration date indicated on the label.
- Results may vary due to variations in blotting conditions.
  Note: *Because VisULite MAX™ ECL Western Blotting Substrate is a highly sensitive substrate, optimization of primary and secondary antibodies may be required to generate consistently reproducible results with strong signals and low backgrounds. See www.rndsystems.com/resources/protocols/western-blot-cell-lysate-protocol for additional optimization tips and hints that may further improve performance.*
- Do not allow membrane to completely dry during procedure.
- Do not use Sodium Azide in any buffers used for detection. Azide is an inhibitor of HRP.
- Work quickly once the membrane has been exposed to VisULite MAX™ ECL Western Blotting Substrate to capture maximum signal.

OTHER MATERIALS REQUIRED

- SDS-PAGE Gel System and Transfer Apparatus
- Nitrocellulose or PVDF Membrane
- Primary and Secondary Antibody
- Blocking Buffer and Wash Buffer
- 0.2M Sodium Phosphate, pH 8.5
- X-Ray Film or Imaging System

REAGENT PREPARATION

Allow VisULite MAX™ ECL Western Blotting Substrate A and Substrate B to equilibrate to room temperature approximately 30 minutes before use. Mix equal volumes of VisULite MAX™ ECL Western Blotting Substrate A and Substrate B. Prepare only the amount of VisULite MAX™ ECL Western Blotting Substrate needed for your experiment.

PROCEDURE

1. Run SDS-PAGE gel and transfer to nitrocellulose or PVDF by established laboratory protocol.
2. Label and block the membrane.
3. Incubate the membrane with primary antibody.
   Note: *High sensitivity of substrate may require adjusting established parameters for primary and secondary antibody.*
4. Wash and incubate with appropriate HRP-labeled secondary antibody.
5. Wash, and for best results, use 0.2 M Sodium Phosphate for the final wash. At the completion of the wash, drain off all liquid and gently blot dry on a lab wipe or filter paper.
   Note: *Do not let membrane dry completely.*
6. Place membrane on clean plastic wrap and add approximately 100 µL of VisULite MAX™ ECL Western Blotting Substrate per square centimeter of membrane. Allow to incubate for 1 minute.
7. Drain excess substrate and gently blot dry as above.
8. Place membrane in CCD camera system and expose according to manufacturer's instructions or place membrane on clean plastic wrap (or equivalent) and expose to film.
9. Adjust exposure times as needed.

DATA EXAMPLES

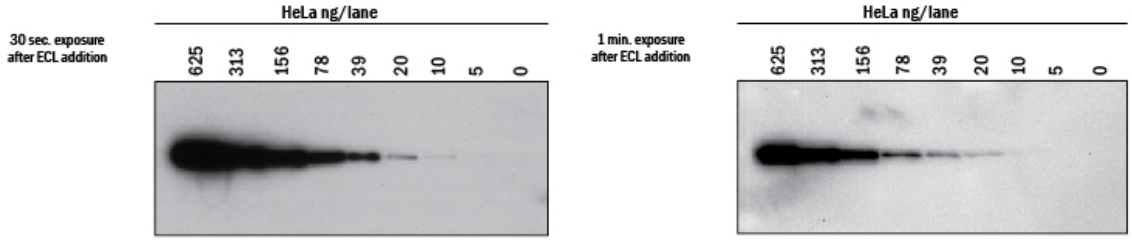

Figure 1: Detection of GAPDH in HeLa Cell Lysates Visualized with VisULite MAX™ ECL Western Blotting Substrate using X-ray film or CCD imaging system. A two-fold dilution series of HeLa whole cell lysate was prepared and loaded at 625, 313, 156, 78, 39, 19, 10 and 5 ng per lane. Samples were transferred onto PVDF membrane and probed with 0.1 µg/mL of Mouse Anti-Human/Mouse/Rat GAPDH Monoclonal Antibody (R&D Systems®, Catalog # MAB5718) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (R&D Systems®, Catalog # HAF018). VisULite MAX™ ECL reagent was applied for 1 minute, followed by exposure to a standard sensitivity film (left) or image capture using ProteinSimple® FluorChem™ M (right). Lanes are visualized on a 30 second exposure (film) or 1 minute exposure (imager).

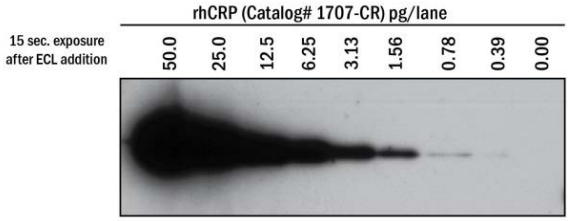

Figure 2: Detection of Recombinant Human CRP Visualized with VisULite MAX™ ECL Western Blotting Substrate. A two-fold dilution series was prepared using Recombinant Human CRP (R&D Systems®, Catalog # 1707-CR) and loaded at 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78, and 0.39 pg per lane. Samples were transferred onto PVDF membrane and probed with 0.5 µg/mL of Sheep Anti-Human CRP (R&D Systems®, Catalog # AF1707) followed by HRP-conjugated Anti-Sheep IgG Secondary Antibody (R&D Systems®, Catalog # HAF016). VisULite MAX™ ECL reagent was applied for 1 minute, followed by exposure to a standard sensitivity film for 15 seconds.

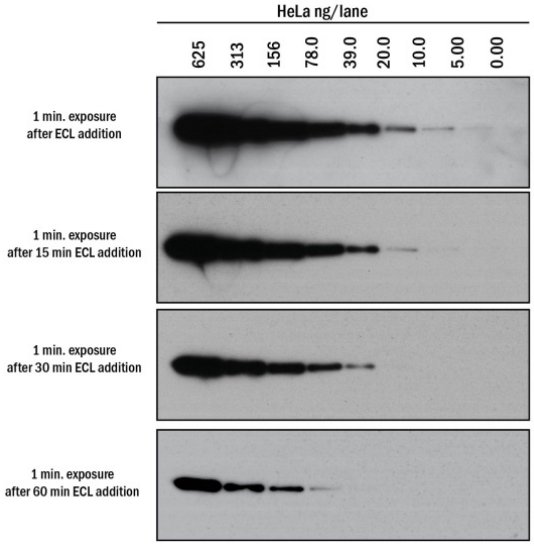

Figure 3: Signal Duration with VisULite MAX™ ECL Western Blotting Substrate. A two-fold dilution series of HeLa whole cell lysate was prepared, loaded, transferred and probed with Mouse Anti-Human/Mouse/Rat GAPDH Monoclonal Antibody (R&D Systems®, Catalog # MAB5718) as in Figure 1 (above). VisULite MAX™ ECL reagent was applied for 1 minute to the PVDF membrane, followed by exposure to a standard sensitivity film for 1 minute at the indicated time points from 1 to 60 minutes after VisULite MAX™ ECL addition.