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## Certificate of Analysis

### Visualizer™ Spray & Glow™ ECL Western Blotting Detection System

Catalog # 17-373SP

Lot # 32300

**Product Description:** One spray bottle containing 40ml of Visualizer™ Spray & Glow™ western blotting detection reagent. The Visualizer™ Spray & Glow™ system is a light emitting non-radioactive method for detection of immobilized specific antigens in chemiluminescent Western blots through horseradish peroxidase (HRP) labeled antibodies. The kit is designed for a high, long lasting glow reaction while combining chemiluminescent substrate and enhancer solutions together in one bottle. There is no mixing of solutions required.

**Storage and Stability:** Stable for 1 year at 4°C from date of shipment.

**Use:** See enclosed protocol. The Visualizer™ Spray & Glow™ Western Blotting Detection System is very sensitive and optimization of the individual assay components (antibodies, conjugates, membranes, etc.) needs to be completed to minimize non-specific reactivities and supply the most robust results.

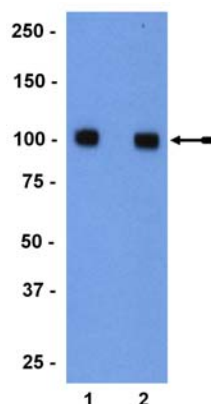
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**FOR IN VITRO RESEARCH USE ONLY  
NOT FOR USE IN HUMANS OR ANIMALS**

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### Quality Control Testing

**Immunoblot Analysis:** The reagents in this kit have been matched to optimize the range and sensitivity of detection using various antibodies in Western blot assays.



#### Immunoblot Analysis

Whole adult rat brain lysate was resolved by electrophoresis, transferred to PVDF and probed with an anti-GluR2 antibody (Chemicon, Catalog # AB1768). Proteins were detected using an HRP conjugated secondary antibody and Visualizer™ Spray & Glow™ (lane 1) or ChemiLucent Detection Kit (Chemicon, Catalog # 2600, lane 2). Arrow indicates GluR2 (~105kDa).

### Visualizer™ Spray & Glow™ Protocol

To achieve the optimal signal to background ratio, it is essential for the end user to optimize concentrations of both primary and secondary antibodies.

1. Remove the PVDF or nitrocellulose membrane from the last wash and place on a flat surface, protein side up. Do not allow the membrane to dry.
2. Spray the Visualizer™ Spray & Glow™ detection reagent on the surface of the membrane.  
**Note:** *Each single spray of Visualizer™ Spray & Glow™ delivers approximately 500 $\mu$ l. Two sprays is usually sufficient to cover a 5cm x 5cm membrane.*
3. Incubate for 1-5 minutes at room temperature without agitation.  
**Note:** *Incubation for one minute is sufficient for detection of specific proteins. Less specific proteins may require slightly longer incubation times.*
4. Drain off excess detection reagent by holding the membrane vertically and touching the bottom edge against absorbent paper.  
**Note:** *This step is important to remove excess background signal.*
5. Wrap the membrane in plastic wrap and gently smooth out air pockets.
6. Place the membrane in a film cassette, protein side up.
7. Place X-ray film on top of membrane, close the cassette and expose for an appropriate time.  
**Note:** *For abundant proteins, 30 seconds to 5 minutes should be sufficient for adequate exposure. More dilute formulations will require longer exposure times. The appropriate exposure time should be determined by the end user.*