GLOBrite Chemiluminescent Reagent Kit For Western Blotting

Catalog Number: **GLB1 and GLB2**

Introduction

The Detroit R & D GLOBrite Chemiluminescent Reagent Kit combines high sensitivity (less than 10 pg following a two minute exposure time) with low cost and easy use in a non-radioactive format. This kit is for detection of immobilized antigens conjugated to horseradish peroxidase labeled antibodies.

Storage and Stability

This kit will obtain optimal results if all of the components are stored at the proper temperature 2 to 8°C prior to use. Items should be stored at the designated temperatures upon receipt of this kit. Use before expiration date.

Materials Provided

Part Number	Item	Description	Quantity
1	Detection Reagent 1	50 mL (GLB1) or 125 mL (GLB2)	2
2	Detection Reagent 2	50 mL (GLB1) or 125 mL (GLB2)	2

Additional Required Materials (Not Provided)

- Film
- Plastic wrap or envelope to insert membrane
- Film developer or imager
- Cassette

Precautions

- Please read all instructions carefully before beginning the assay.
- The reagents in this kit have been tested and formulated to perform optimally. This kit may not perform correctly if any of the reagents are replaced or any of the procedures are modified.
- This kit is intended for research use only and is not to be used as a diagnostic.

Protocol for Developing Membranes Following Transfer and Incubation with Primary and Secondary Antibodies

1) An amount of ECL reagent that is sufficient to cover the membrane should be prepared.
2) Use mixed reagents immediately after preparation.
3) Mix equal parts of Reagent 1 and Reagent 2
4) Incubate membrane in ECL reagents for 5 minutes at room temperature.
5) Remove membrane and drain excess ECL reagents from membrane
6) Place membrane in plastic sheet (protein side up).
7) If using film, expose membrane to film in a dark room and place in a film cassette
8) Incubate for appropriate amount of time before developing. Optimum incubation time is dependent on primary and secondary antibodies used. It is recommended that the first exposure be of 1 minute duration. Adjustments in exposure time can then be made based upon this initial exposure.
9) If using a film imager, place membrane inside imager following step 5 and expose for appropriate length of time.

Troubleshooting

No signal or very weak signal

- Concentration of primary antibody used is too low. Lower dilution of primary antibody.
- Protein did not transfer correctly to membrane. Make sure that transfer apparatus was assembled correctly. Increase length of transfer time.
- Insufficient protein was loaded on gel. Load more protein in well.
- Length of exposure of membrane with film was too short. Increase exposure time.

The background is very high.

- Concentration of primary or secondary antibody is too high. Further dilute primary or secondary antibody.
- Incompatible or insufficient blocking agent was used. Change blocking agent or adjust Tween 20 concentration in buffers.
- Insufficient washing of membrane between and after exposures to primary and secondary antibodies. Include more washes or agitate membranes more strongly during washing.
- Exposure of membrane to film was too long. Reduce exposure time.

References

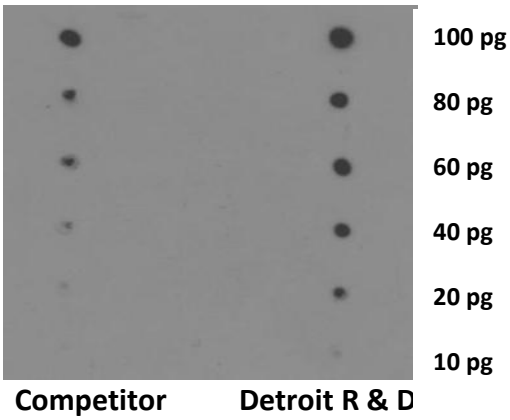
Towbin, H. and Gordon, J. (1984). Immunoblotting and dot immunoblotting – Current status and outlook. J.Immunol. Meth. 72:313-40.

Young, P.R. (1989) An improved method for the detection of peroxidase conjugated antibodies on immunoblots. J. Virol. Meth. 24:227-36.

Warranty

Shelf Life: 1 year at 4°C

Detroit R&D, Inc., makes no warranty of any kind expressed, or implied, including, but not limited to the warranties of fitness for a particular purpose and merchantability.



Comparison of Detroit R & D Chemiluminescent Reagent Kit versus a competitor's kit following a two minute exposure time.

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