ProtoGlow ECL

national diagnostics

Enhanced Chemiluminescent Substrate

- Extended signal life
- Long shelf life/extra reliability
- Less antibody needed

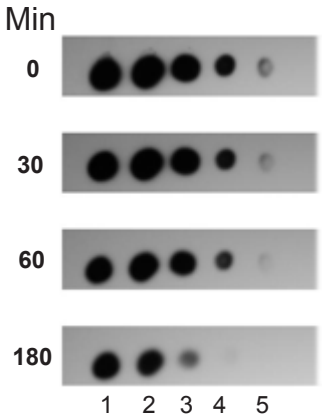
National Diagnostics' ProtoGlow ECL delivers the latest technology developed for enhanced chemiluminescent detection on Western blots. The unique chemistry of the ProtoGlow ECL system increases the sensitivity of Western blots by up to 20 fold. This allows the detection of proteins at much lower abundance and/or the use of higher dilutions of primary and secondary antibodies, economizing on these expensive reagents.

The improved sensitivity of ProtoGlow ECL is demonstrated below. Transferrin was separated, transferred, blocked and probed, then subjected to detection using ProtoGlow ECL:

Gel electroblotted onto nitrocellulose and probed with HRP-labelled antibody at 1:30,000 dilution. Detection using ProtoGlow ECL. Blot was exposed for 90 seconds. Lane 1) 100 ng; Lane 2) 50 ng; Lane 3) 25 ng; Lane 4) 10 ng; Lane 5) 5 ng.

LONG LASTING SIGNAL

ProtoGlow ECL generates a consistent light output for as long as 120 minutes. This ensures results are more reproducible exposure to exposure, blot to blot. It also allows multiple exposures of a single blot to be taken to optimize the signal:noise ratio.



Sequential exposures of serially diluted HRP conjugated anti-transferrin antibody detected with ProtoGlow ECL. Exposures of 60 seconds were taken at 0, 30, 60 and 180 minutes. Dilutions: Lane 1) 1:10,000; Lane 2) 1:20,000; Lane 3) 1:40,000; Lane 4) 1:80,000; Lane 5) 1:160,000.

ECONOMICAL

ProtoGlow ECL allows the researcher to use less antibody. The extremely enhanced signal from ProtoGlow ECL allows you to use anywhere from 4 to 40 times less antibody per blot while retaining the same detection sensitivity. This economizes on the consumption of expensive antisera.

STORAGE

ProtoGlow ECL kit components are best stored refrigerated (4 °C). ProtoGlow ECL is stable for up to one (1) year.

ProtoGlow ECL	CL-300
200 ml kit	
500 ml kit	

METHOD OF USE

ProtoGlow ECL fits into any existing Western blot workflow using the same basic protocol as standard ECL kits. *Note:* The extreme sensitivity of this substrate may require additional dilution of antibody stocks and/or alterations in blocking protocols to avoid excessive background.

DETAILED PROTOCOL

1. Prepare your protein blot. PVDF or nitrocellulose may be used.

2. Block membrane for 60 minutes at room temperature. Use BLOTTO, BSA or nonprotein blocking agents.

3. Incubate blot with primary antibody for 60 minutes at RT with gentle agitation.

4. Wash blot in PBST or TBST as follows:

   a) One brief wash

   b) Once at 15 minutes with 1.0 mL/cm^2 membrane

   c) Three times for 15 minutes with 0.5 mL/cm^2 membrane per wash

5. Incubate blot with secondary antibody for 60 minutes at RT on an oscillating plate or rocker.

6. Wash blot three times in TBST for 5 minutes each with at least 0.5 mL/cm^2 membrane per wash.

7. Make the ProtoGlow ECL reagent by mixing equal amounts of parts A and B. Provide 0.1-0.2 mL/cm^2 membrane and place on blot for 2 minutes.

8. Drain excess reagent.

9. Cover damp blot with plastic wrap and place in cassette.

10. Insert film into cassette and expose. In most cases, 10-60 seconds is a sufficient exposure.

TROUBLESHOOTING

Problem: No bands or faint bands

Solutions:

- Check activity of secondary antibody (use a dot blot for a quick test)
- Check that secondary antibody recognizes the primary. Probe a dot blot of the primary with the secondary antibody. Use BSA as a negative control.

Problem: High background

Solutions:

- Reduce primary and/or secondary antibody concentration
- Use a shorter exposure time.
- Try a different blocking buffer. Some antibodies react with skim milk or BSA.
- Reduce concentration of blocking agent.
- Increase washing time.
- Unwrap blot, wash for 60 seconds in TBST and re-expose.

Problem: Speckled background

Solutions:

- Use only powder-free gloves.
- Filter antibody and blocking solutions.
- Cover the tray while processing the blot to exclude dust.

Ordering Information For ProtoGlow ECL and accessories:

ProtoGlow ECL 200 mL kit 500 mL kit	CL-300
ProtoGel (30%) 450 ml 1 L	EC-890
ProtoGel (40%) 450 mL 1 L	EC-891
10X Tris-Glycine Electroblotting Buffer 1 L 4 L	EC-880
10X Tris-Glycine-SDS PAGE Buffer 1 L 4 L	EC-870
10X TBS 1 L	EC-881
10X TBST 1 L	EC-882

For more information or order placement:

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