MagicBrite ECL DuraPlus Chemiluminescent Substrate

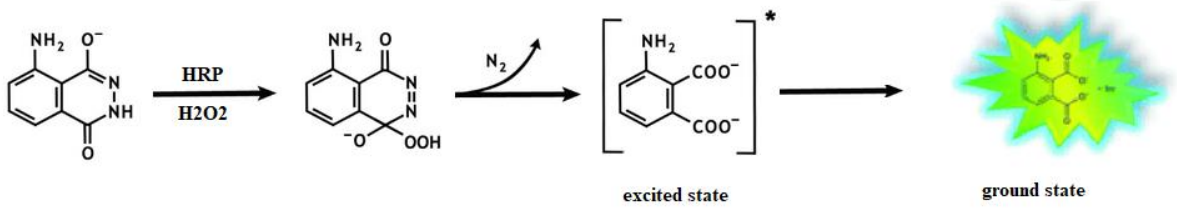
Components

Components		Product size		Storage
		BP-50070	BP-50071	
Solution A	Luminol/enhancer solution (amber bottle)	10 ml	100 ml	Room temperature
Solution B	StablePeroxide solution (white bottle)	10 ml	100 ml	Room temperature

Overview

MagicBrite ECL DuraPlus Chemiluminescent Substrate is a sensitive, luminol-based enhanced chemiluminescent substrate for detecting horseradish peroxidase (HRP) activity on immunoblots. MagicBrite ECL DuraPlus Chemiluminescent Substrate enables low picogram or high femtogram detection of antigen by oxidizing luminol in the presence of HRP and peroxide. This reaction produces a prolongedchemiluminescence that can be visualized on X-ray film or an imaging system.

BroadPharm's MagicBrite ECL DuraPlus Substrate provides excellent performance, versatility and long-lasting signal output for publication quality results.



Optimal signal intensity and duration can be attained with appropriate primary and secondary antibody dilutions (see Table 1).

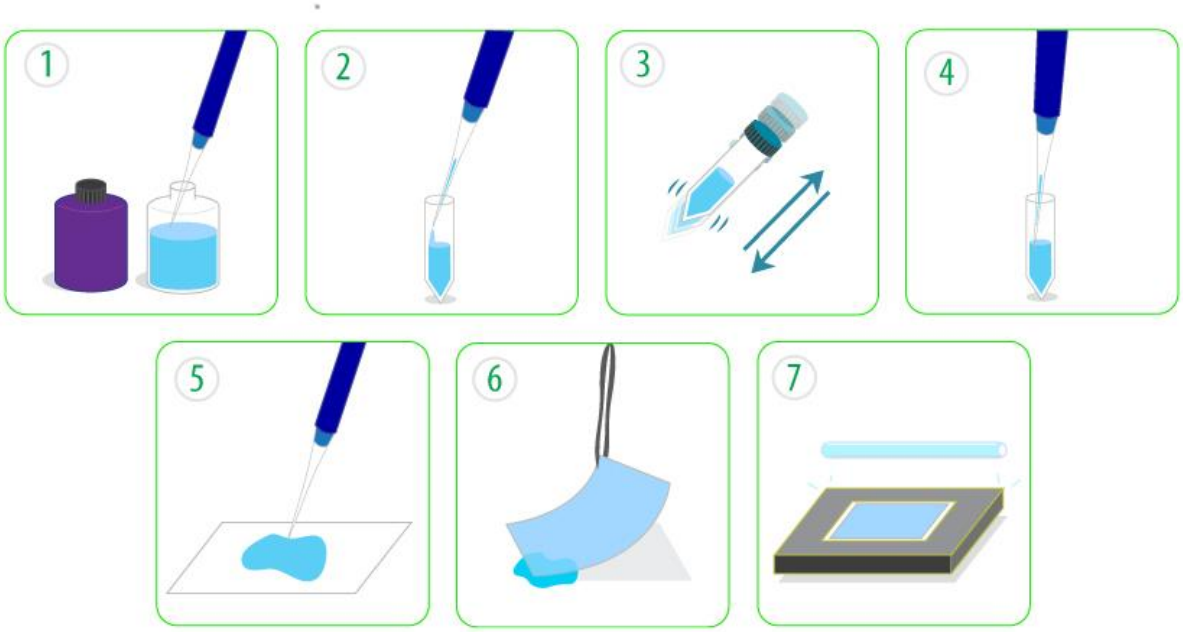
Table 1 Antibody dilution ranges to use with MagicBrite ECL DuraPlus Chemiluminescent Substrate.

Primary antibody dilution ranges from a 1 mg/ml stock	Secondary antibody dilution ranges from a 1 mg/ml stock
1:1,000-1:50,000 or follow vendor's recommendation dilution range.	1:50,000-1:250,000 or follow vendor's recommendation dilution range.

Guideline

- Good western blot results require optimizing the process components and steps; including sample amount, gel type, transfer method, membrane type, blocking solution, wash buffer, primary antibody concentration, secondary antibody concentration, and incubation times.

- Use a sufficient volume of all solutions to ensure the membrane never becomes dry.
- For optimal results, use a shaking or rocking platform during incubation steps.
- Do not use sodium azide as a preservative for buffers, as it inhibits HRP activity.
- Always wear gloves and use clean, plastic forceps. Metallic devices (e.g., scissors) must have no visible signs of rust, which may cause speckling and/or high background.
- The substrate working solution is stable for 8 hours at room temperature. Exposure to the sun or any other intense light can harm the working solution. However, short-term exposure to laboratory lighting is not an issue.



Materials and equipment required but not included:

- Electrophoresis apparatus and buffers for SDS-PAGE.
- Transfer apparatus for protein transfer from gel to western blot membrane. Use any suitable protocol to separate proteins by electrophoresis and transfer them to a membrane.
- Rotary or rocking platform shaker for agitation of membrane during incubations.
- Nitrocellulose or PVDF membrane.
- Blocking buffer (Tris buffered saline (TBS) or Phosphate buffered saline (PBS) with 0.05-0.1% Tween-20 and 1-5% of a blocking reagent, such as bovine serum albumin (BSA), gelatin, casein, non-fat dry milk).
- Washing buffer (TBS or PBS with 0.05-0.1% Tween-20).
- Primary antibody compatible with your application.
- Secondary antibody, conjugated with horseradish peroxidase (HRP) corresponding to your primary antibody.
- X-ray film or a CCD-based imager.

Protocol for Western Blot

- After protein had transferred to a blotting membrane, block the membrane for 1 hour at room temperature, or overnight at 4°C using blocking buffer.
- Incubate the membrane with dilute primary antibody, room temperature or 37°C for 1 hour, or 4°C overnight.
- Wash the membrane in wash buffer (TBST) 3x, 5 minutes each.
- Incubate the membrane with dilute conjugated secondary antibody at room temperature for 30 minutes to 1 hour.
- Wash the membrane in wash buffer 3x, 5 min. each.
- Signal development using MagicBrite ECL DuraPlus Chemiluminescent Substrate, follow the kit's instruction. (Reference diagram for ECL preparation and use).
- Prepare Working Solution by mixing equal parts of the Stable Peroxide Solution and the Luminol/Enhancer Solution. Use 0.1 mL working solution per cm^2 of membrane. The working solution is stable for 8 hours at room temperature. (Diagram for ECL prep, steps 1 – 3).
- Incubate the blot in working solution for 5 minutes. (Diagram for ECL prep, step 4 and 5).
- Remove the blot from working solution and drain excess reagent.
- Place the blot in clear plastic wrap or sheet protector and remove bubbles. (Diagram for ECL prep, step 6).
- Expose the blot to X-ray film or imaging system. (Diagram for ECL prep, step 7).

Product Data

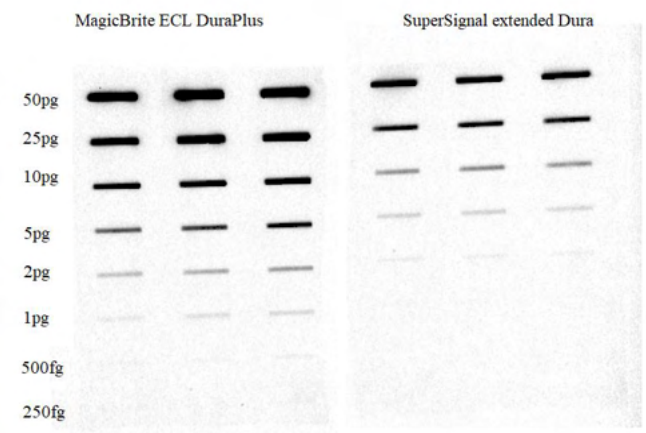


Figure 1
MagicBrite ECL DuraPlus detection of HRP conjugated antibody by slot-blot:

Rabbit anti-goat-HRP antibody (catalog # BP-50081) was immobilized on a nitrocellulose membrane and detected with MagicBrite ECL DuraPlus and SuperSignal extended Dura in combination with an imaging system (ChemiDoc XRS+), exposure at 180 seconds.

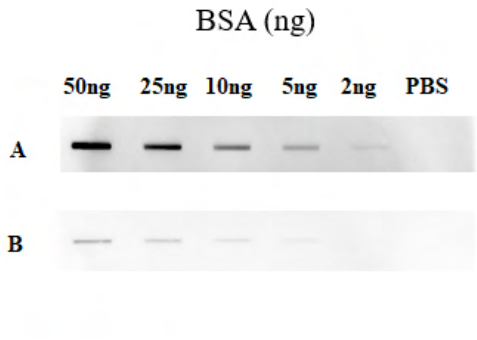


Figure 2
Slot blot visualization with MagicBrite ECL DuraPlus vs. SuperSignal extended Dura:

The indicated amount of BSA (50ng to 2ng) was immobilized on a nitrocellulose membrane and incubated with MagicBrite DuraPlus ECL Substrate (A) and SuperSignal extended Dura(B) prior to chemiluminescent detection (anti-BSA goat IgG: 1:2000; rabbit anti-goat HRP antibody: 1:80K, ChemiDoc XRS+, 4 seconds exposure). All experiments were performed in duplicate.

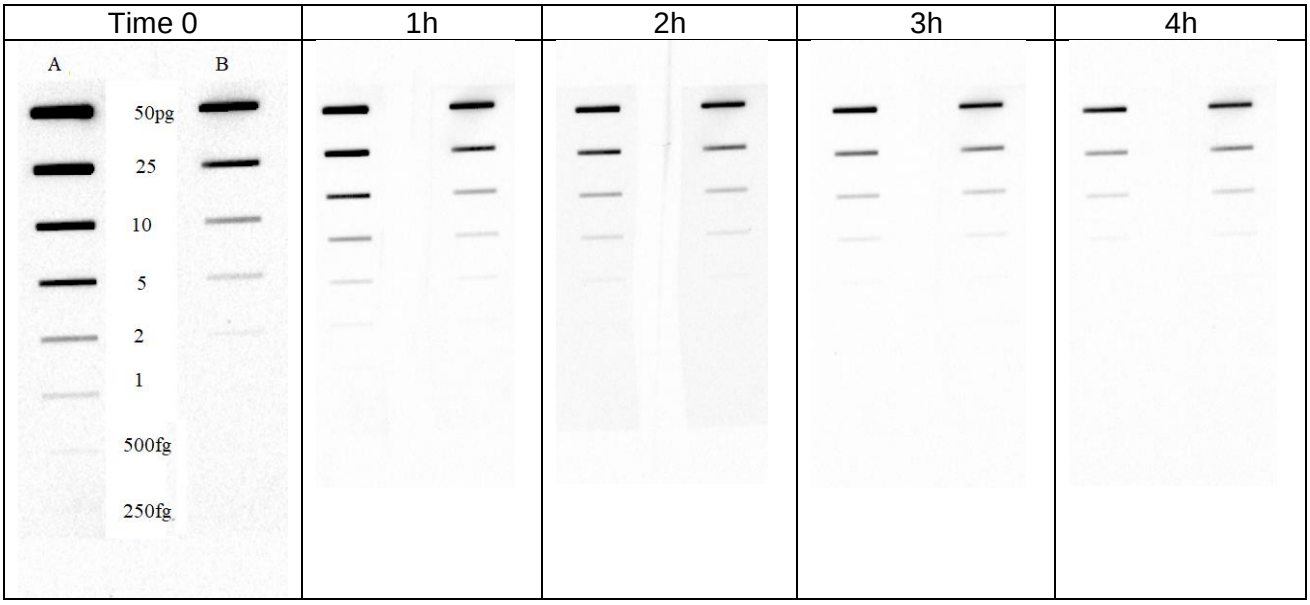


Figure 3
Signal duration comparison by slot-blot:
Rabbit anti-goat-HRP antibody (catalog # BP-50081) was immobilized on a nitrocellulose membrane and detected with MagicBrite ECL DuraPlus, and SuperSignal extended Dura with an imaging system at various time points.

Troubleshooting

Observation	Possible cause	Recommended action
Reverse image on film (i.e., white bands on black background].	Excess HRP in the system	Further dilute the HRP-conjugate
Membrane has brown or yellow bands.	Excess HRP in the system	Further dilute the HRP-conjugate
Blot glows in the darkroom.	Excess HRP in the system.	Further dilute the HRP-conjugate
Signal rapidly dies.	Excess HRP in the system.	Further dilute the HRP-conjugate Or Load less sample.
Weak or no signal.	Excess HRP in the system depleted the substrate and caused the signalto quickly fade.	Further dilute the HRP-conjugate
	insufficient quantities of antigen orantibody.	Increase amount of antibody or antigen.
		Use MagicBrite ECL DuraPlus substrate
	Inefficient protein transfer.	Optimize transfer.
	Contamination	All the devices and equipment or reagents should be cleaned carefully. Some impurity even at ppm level can quench the reaction. Use clear devices and try different source of HRP to determine the root cause.
High background.	Excess HRP in the system.	Further dilute the HRP-conjugate
	inadequate blocking.	Optimize blocking conditions
	inappropriate blocking agent.	Try a different blocking agent
	inadequate washing.	Increase length, number, or volume of washes.

	Overexposed film.	Decrease exposure time
	Antigen or antibody concentration too high.	Decrease amount of antigen or antibody.
	Poor antibody specificity.	Try different source of HRP to determine the root cause
Spots within protein bands.	inefficient protein transfer.	Optimize transfer procedure.
	Unevenly hydrated membrane.	Perform manufacturer's recommendations for properly hydrating membrane.
	Bubble between the film and membrane	Remove bubbles before exposing blot to film
Speckled background on film.	Aggregate formation in the HRP-conjugate.	Filter conjugate through a 0.2 µm filter.
Nonspecific bands.	Excess HRP in the system.	Further dilute the HRP-conjugate
	SDS caused nonspecific binding to protein bands.	Do not use SDS during the Western blot procedure.
	Poor antibody specificity.	Try different source of HRP to determine the root cause
	insufficient blocking.	Increase blocking time or use different blocking reagent