Troubleshooting & FAQ

Problem	Possible Cause	Possible Cause
Weak signal	Insufficient quantity of sample	Load a higher concentration of sample onto the gel
	Poor protein transfer	Optimize transfer conditions
	Insufficient quantity of primary Ab was used	Strip and re-probe the blot using a higher amount of primary Ab
	Weak antibody-antigen affinity	Use alternate protocol for enhanced sensitivity
High background	Insufficient washing	Use 25mL of Eaze-Wash Buffer per mini blot in a suitable incubation tray. Add one additional wash step using 25mL of 1X PBST if background persists.
	Excessive quantity of primary Ab was used	Repeat WesternEaze-Chemi using a lower amount of primary Ab
Artifacts on blot	Speckling	Ensure that the gel and membrane are not over-heated during electrophoresis or transfer
	Poor protein transfer	Optimize transfer conditions
	Uneven or blotchy background	Use a shaking incubator for all steps. Make sure that the membrane is wet throughout the entire process. Avoid touching the membrane and always wear clean gloves. Do not stack blots during incubation or washing steps. Ensure that all equipment and incubation trays are clean and free of contaminating particulates.
	Bubbles in between the membrane and the gel	Use a roller to carefully remove all air bubbles

For More Information

visit **www.advansta.com/products/WesternEaze-Chemi** or go directly to the web-page by scanning the QR-code with your favorite bar-code scanner app on your smart phone.

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WesternEaze-Chemi Kit

Rapid, high sensitivity chemiluminescent Western blot kit for protein detection

For Catalog Numbers

K-12054-010 Anti-Mouse, sufficient for 10 blots
K-12055-010 Anti-Rabbit, sufficient for 10 blots

Description

The WesternEaze-Chemi Kit includes all critical reagents required to generate high sensitivity chemiluminescent Western blot data for 10 mini blots in one hour. The blocking buffer is specially formulated to increase sensitivity while decreasing overall background. The unique HRP Conjugate amplifies signal to increase sensitivity while minimizing the duration of incubation. The wash solution contains a gentle, non-ionic detergent that rapidly removes non-specifically bound material from the membrane without disrupting the antibody-antigen complex. WesternBright™ ECL substrate is included for sensitive, long-lasting and flexible detection using X-ray film or CCD camera. Provided as ready-to-use solutions designed to simplify your Western blot workflow.

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May 3, 2019 | D-26042-408

Storage Information

The WesternEaze-Chemi Kit is shipped at room temperature. Upon receipt, store each component at designated temperature.

- R-03733-D10 Buffer A, Eaze-Blocking/Incubation Buffer, 100mL 2°–4°C
- R-03734-500 Buffer B, Mouse Eaze-HRP Conjugate, 500µL 2°–4°C
- R-03735-500 Buffer B, Rabbit Eaze-HRP Conjugate, 500µL 2°–4°C
- R-03736-D25 Buffer C, Eaze-Wash Buffer, 250mL Room temperature
- R-03031-C50 WesternBright™ ECL Substrate, 50mL Room temperature
- R-03025-C50 WesternBright™ ECL Peroxide, 50mL Room temperature

Warnings and Precautions

- The WesternEaze-Chemi Kit is for research use only.
- Always wear gloves when handling membranes and reagents.
- Refer to SDS for additional safety information.
- The product is guaranteed to be free of manufacturer defects, and to function as described when the provided protocol is followed by properly trained personnel.

Short Protocol (1 Hour)

1. Prepare your protein blot on either PVDF or Nitrocellulose using your standard technique.
2. Following the transfer, immerse the blot in 10mL of Buffer A, Eaze-Blocking/Incubation Buffer, ensuring that the entire surface of the blot is completely covered.
3. Incubate the blot for 15 minutes with gentle agitation.
4. Add 5µg of primary antibody immediately followed by 50µL of Buffer B, Mouse or Rabbit Eaze-HRP Conjugate directly to the solution containing the blot.
5. Incubate for 30 minutes with gentle agitation.
6. Decant the solution from the blot.
7. Add 25mL of Buffer C, Eaze-Wash Buffer to the blot and incubate for 10 minutes with gentle agitation.
8. During the wash step, prepare a working solution of chemiluminescent substrate.
   - Mix components 1 and 2 in a 1:1 ratio in sufficient amounts to obtain at least 0.1 ml/cm^2 of the blot.
9. Thoroughly drain all wash solution from the blot then apply the working solution of chemiluminescent reagent to the blot (use 0.1mL/cm^2 of your membrane). Incubate the blot with the reagent for 5 minutes.
10. Drain excess substrate and place the blot in your CCD imager and image. If a long exposure is required or if imaging will be performed using X-ray film, place the blot in a blot development folder for best results.

Alternate Protocol for Enhanced Sensitivity (1.5 Hours)

1. Prepare your protein blot on either PVDF or Nitrocellulose using your standard technique.
2. Following the transfer, immerse the blot in 10mL of Buffer A, Eaze-Blocking/Incubation Buffer, ensuring that the entire surface of the blot is completely covered.
3. Incubate the blot for 15 minutes with gentle agitation.
4. Add 5µg of primary antibody directly to the solution containing the blot.
5. Incubate for 30 minutes with gentle agitation.
6. Add 50µL of Buffer B, Mouse or Rabbit Eaze-HRP Conjugate directly to the solution containing the blot and primary antibody.
7. Incubate for 30 minutes with gentle agitation.
8. Decant the solution from the blot.
9. Add 25mL of Buffer C, Eaze-Wash Buffer to the blot and incubate for 10 minutes with gentle agitation.
10. During the wash step, prepare a working solution of chemiluminescent substrate.
    - Mix components 1 and 2 in a 1:1 ratio in sufficient amounts to obtain at least 0.1 ml/cm^2 of the blot.
11. Thoroughly drain all wash solution from the blot then apply the working solution of chemiluminescent reagent to the blot. Incubate the blot with the reagent for 5 minutes.
12. Drain excess substrate and place the blot in your CCD imager and image. If a long exposure is required or if imaging will be performed using X-ray film, place the blot in a blot development folder for best results.