

CHEMILUCENT PLUS WESTERN BLOT ENHANCING KIT TRIAL KIT

CATALOG NUMBER: 2650-S

LOT NUMBER:

QUANTITY: 1 Kit (1,200 cm²)

DESCRIPTION: The Chemilucient Plus can be used independently to boost the signal obtained with all ECL based Western blot kits. Chemilucient Plus is derived from a powerful, innovative proprietary technology. Unlike conjugated complex- or chemiluminescent substrate-enhancers, Chemilucient Plus works through a novel mechanism by increasing antigen and antibody affinity and accessibility. Due to its unique mechanism, Chemilucient Plus can work with different antigens and antibodies, such as structural proteins (Actin), enzymes (GAPDH) or membrane proteins (EGFR). It could significantly boost Western blot signals, and work together with the two other kinds of enhancing mechanisms to achieve multiplied enhancing effects.

KIT COMPONENTS:

Chemilucient Plus Reagent	50 mL
Antibody Binding Buffer (20X)	60 mL
Chemilucient Plus Blocking Reagent	20 grams

MATERIALS REQUIRED BUT NOT SUPPLIED:

- Primary antibody
- Secondary antibody
- Chemiluminescent Substrates (Catalog number 2600)
- Wash buffer (1X TTBS)
- Membrane stripping and re-probing buffer (Catalog number 2500)

REAGENT PREPARATION:

1X TTBS
Add 6.05 g Tris base (50 mM), 8.76 g sodium chloride (150 mM) to 800 mL of distilled water. Adjust pH to 7.5 with HCl. Adjust to 1 liter with distilled water. Add Tween-20 to 0.1% (v/v). Maybe stored for up to 3 months at room temperature.

Blocking Reagent

Weight 5 g of Chemilucient Plus Blocking Reagent and dissolve it in 100 mL of 1X TTBS solution. Make fresh daily.

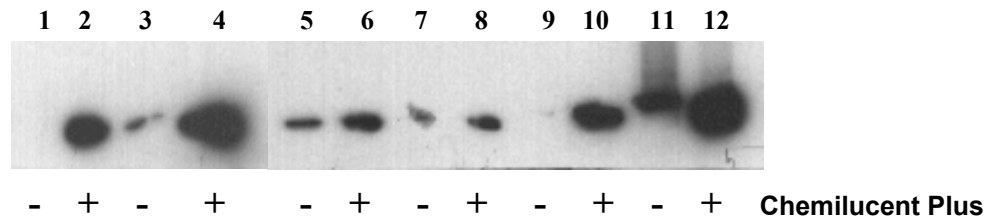
PROTOCOL:

1. Remove blot from the transfer apparatus and soak in transfer buffer. Under the hood, pour the Chemilucient Plus solution into a new container. For a 50 cm² blotting membrane use 10 mL of Chemilucient Plus. The solution can be reused up to 4 times without losing its enhancing effect. Remove the membrane from the transfer buffer and drain the remaining buffer on the membrane. For the best results do not let the membrane dry completely. Soak the membrane in the Chemilucient Plus solution under agitation for 2 minutes then remove the membrane and submerge it in 1X TTBS.
2. Block the membrane with 5% non-fat dry milk in 1X TTBS solution for 30 minutes at room temperature under agitation.
3. Prepare the Antibody Binding Buffer by diluting the supplied 20X stock solution 20 times with ddH₂O. Make an appropriate dilution of your primary antibody in the 1X Antibody Binding Buffer. 10 mL of primary antibody solution is suggested for a 50 cm² membrane. Incubate the blot for one hour at room temperature to overnight at 2-8C.

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4. Wash the membrane in 1X TTBS for 5 minutes. Repeat 3-4 times.
5. Incubate the blot with the appropriate HRP-conjugated secondary antibody solution diluted with the Antibody Binding Buffer for one hour at room temperature. 10 mL of secondary antibody solution is recommended for a 50 cm² membrane.
6. Repeat step 4 to remove unbound HRP-conjugate.
7. Continue using the directions from your ECL kit (for example Chemicon catalog number 2600).

SAMPLE RESULTS:



Western blot of total protein extracts from human placenta incubated with an antibody against GAPDH and developed using commercially available chemiluminescent detection systems from various sources. Compare unenhanced Chemilucifer signals (-) with signals enhanced using Chemicon's Chemilucifer Plus signal enhancing reagent (+). Lanes 1-2: Company A; Lanes 3-4: Company B; Lanes 5-6: Company C, Lanes 7-8: Company D, Lanes 9-10: Company E; Lanes 11-12: Company F.

STORAGE/HANDLING: Store the kit at 2-8°C for up to 12 months after date of receipt.

Manufactured for Chemicon by Biochain Institute, Inc.

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