BioBharati LifeScience Pvt. Ltd.

ECL Reagent

Cat# BB-C0015 (100 reactions)

Description:

Biobharati ECL Western Blotting detection reagent is a highly sensitive nonradioactive, enhanced luminol-based chemiluminescent substrate for the detection of horseradish peroxidase (HRP) on immunoblots. BBL ECL Western Blotting Substrate enables the detection of picogram amounts of antigen and allows for easy detection of HRP using photographic or electronic imaging methods.

The reagent is characterized by greatly increased signal stability, which allows repeated exposures and makes it easier to process several blots in one experiment.

Important Product Information:

- Exposure to the sun or any other intense light can harm the substrate. Short-term exposure to typical laboratory lighting will not harm the working solution.
- Do not use sodium azide as a preservative for buffers. Sodium azide is an inhibitor of HRP and could interfere with this system.
- Avoid using milk as a blocking reagent when using avidin/biotin systems because milk contains variable amounts of endogenous biotin, which causes high background signal.
- Tween-20 can cause high background as it can interfere with the ECL reagent. Rinsing the membrane briefly with Milli-Q or double-distilled water just prior to ECL detection may alleviate high background. It should be noted that a membrane rinsed with water tends to air-dry more quickly than one that was rinsed with wash buffer so that the ECL detection reagent should be prepared promptly. Otherwise normal TBS buffer without Tween20 can be used as final wash buffer before ECL detection.
- High background can also be alleviated by briefly re-blocking the membrane with non-fat milk prior to incubation in secondary antibody. Milk concentration and blocking time should be determined empirically, but 5–10% milk (w/v) for 30-60 min at R.T. with gentle agitation usually works well for many of the primary antibodies we had studied. This should be followed by a brief washing step prior to incubation in secondary antibody.
- Do not handle membrane with bare hands. Always wear gloves or use clean forceps.

Western Blotting Procedure:

1. Remove blot from the transfer apparatus and block nonspecific sites with Blocking Reagent for 60 minutes at room (Blocking reagents 5% non fat milk or BSA in TBS-T or PBS-T) temperature (RT) with shaking. If desired, block overnight at 2-8˚C without shaking.

2. Remove the Blocking Reagent and add the primary antibody working dilution. Incubate blot for 1 hour at RT with shaking or overnight at 2-8°C with shaking (Antibody Dilution buffer: 1% non fat milk or BSA in TBS-T or PBS-T).

3. Briefly rinse membrane in Wash Buffer (TBS-T or PBS-T) two times.

4. Wash membrane by suspending it in Wash Buffer and agitating for 5 minutes. Replace Wash Buffer at least 4-6 times. Increasing the Wash Buffer volume, the number of washes and wash duration may help minimize background signal.

5. Incubate blot with the HRP-conjugate (secondary antibody) working dilution (Antibody Dilution buffer: 1% non fat milk or BSA in TBS-T or PBS-T) for 1 hour at RT with shaking.

6. Repeat Steps 3 and 4 to remove unbound HRP-conjugate.

Note: Membrane MUST be thoroughly washed after incubation with the HRP-conjugate.

7. Prepare the substrate working solution by mixing equal parts of Detection Reagents (Solution 1 and 2) in 1:1 ratio.

Note: For best results prepare working solution immediately before use. The working solution is stable for 1 hour at RT.

8. Incubate blot with working solution for 1 minute at RT.

9. Remove blot from working solution and place it in a plastic sheet protector or clear plastic wrap. Use an absorbent tissue to remove excess liquid and to carefully press out any bubbles from between the blot and the membrane protector.

10. Place the protected membrane in a film cassette with the protein side facing up. Turn off all lights except those appropriate for X-ray film exposure (e.g., a red safelight).

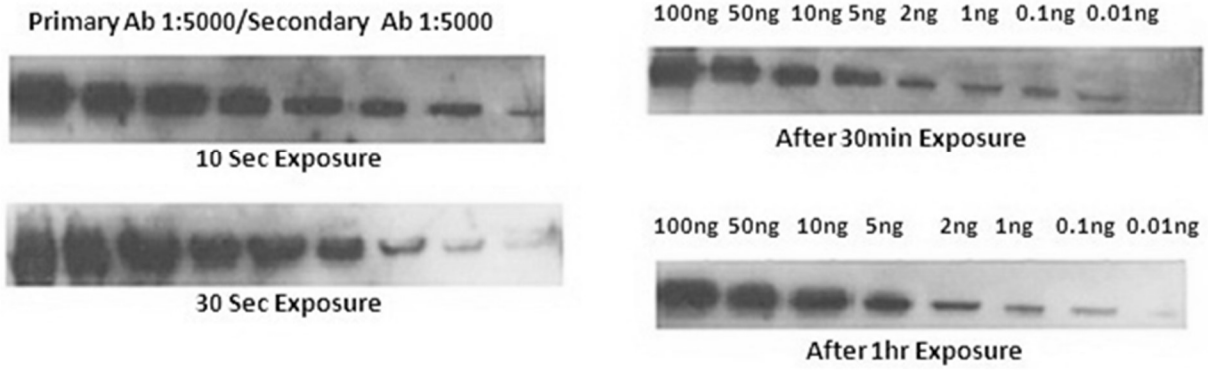
Note: Film must remain dry during exposure. For optimal results, perform the following precautions:

• Make sure excess substrate is removed from the membrane and the membrane protector.

• Use gloves during the entire film-handling process.

11. Carefully place X-ray film on top of the membrane. Perform a first-time exposure of 30 seconds. Vary the exposure time to achieve optimal results.

12. Develop film using appropriate developing solution and fixative. If signal is too intense, reduce exposure time.



Reagents Supplied within this Kit: (Store at 4°C)

ECL Solution A (1ml), ECL Solution B (1ml) and ECL Solution C (0.5ml) supplied as stock Solution.

Preparation of ECL Working Solution: (Prepare the solution just before use)

Take two 2ml eppendorf tube and marked as Solution 1 and Solution 2.

Preparation of Solution 1 (2ml):

Add 1M Tris-Hcl (pH-8.8): 0.1ml
Add ECL Solution A: 10µl
Add ECL Solution B: 5µl
Adjust the volume up to 2ml with MilliQ water. Cover the tube with silver foil. Mix well before use.

Preparation of Solution 2 (2ml):

Add 1M Tris-Hcl (pH-8.8): 0.1ml
Add ECL Solution C: 3µl
Adjust the volume up to 2ml with MilliQ water. Cover the tube with silver foil. Mix well before use.