MBI Evolution Borealis plus Western Blot Detection System

RUO *Research Use Only* REF *16024* *(100 ml each)* 2°C 8°C

DESCRIPTION

The **MBI Evolution Borealis plus Western Blot Detection System** was designed to make western detection of proteins easier by providing the high level of sensitivity with the less amount of background in a chemiluminescent reagent system which has become one of the most common protein analysis techniques used in biochemical research.

The **MBI Evolution Borealis plus Western Blot Detection System** utilizes an enhanced version of the chemiluminescent HRP conjugates. That results in a more sensitive detection of an antigen while providing a long lasting signal and the strong signal to noise ratio of any competitor's.

CHARACTERISTICS

- **Simple** : MBI Evolution Borealis plus Kit is easy to use and designed for high quality, long lasting chemiluminescent reaction
- **Fast** : Specific protein detection may be achieved in less than 1-10 minute.
- **High sensitivity** : MBI Evolution Borealis plus Kit is able to detect picograms level of antigen on membrane blot.
- **High resolution** : MBI Evolution Borealis plus Kit generate high contrast signals

KIT CONTENTS

Component	Amount
MBI Evolution Borealis plus Substrate Solution	100 ml
MBI Evolution Borealis plus Enhancer Solution	100 ml
Instruction Manual	1

STORAGE

- **Storage** : On receipt all components should be stored at 2 – 8°C. The MBI Evolution Borealis plus Western Blot Detection System is sensitive to light. Long term storage of the individual reagent should be in the light blocked containers in which they are provided.
- **Expiry** : The components of MBI Evolution Borealis plus Western Blot Detection System are stable for at least 1 year when stored under the recommended conditions

ADDITIONAL REQUIRED EQUIPMENT

- **Western blot membrane** : Nitrocellulose membrane or PVDF membranes (0.22µm pore size).
- **Dilution Buffer** (TBS) or (PBS).
- **Wash Buffer** : TBS-T or PBS-T (TBS or PBS + 0.05% Tween 20)
- **Blocking Reagent** : 5% non-fat dried milk in TBS-T or PBS-T (Gelatin, Casein and Bovine Serum Albumin (BSA) may also be used as alternative blocking reagents)
- **Primary antibody** : Extremely variable, from 1:10 - 1:100,000. The optimal dilution to use depends on the specific primary antibody and the amount of antigen on the membrane.
- **HRP-conjugated secondary antibody** : commonly working dilution at 10-50 ng/ml (i.e., 1:20,000 - 1:100,000 from a 1 mg/ml stock). The optimal dilution to use varies depending on the specific conjugate and the amount of antigen on the membrane.
- **Film, Film cassette, developing and fixing reagents** or **Imaging System**
- **Rotary platform shaker** : For agitation of membrane during incubations.

PROTOCOLS

◈ Electrophoresis and blotting

1. **Perform electrophoresis and blotting according to usual techniques.**

◈ Block the membrane

1. **Block non-specific binding sites by immersing the membrane in 5% non-fat dried milk, 0.05% (v/v) Tween 20 in PBS or TBS (PBS-T or TBS-T) for 1 hour at room temperature on an orbital shaker. Alternatively, membranes may be left in the blocking solution overnight in a refrigerator at 2–8°C.**

Note : The combination of non-fat dried milk and Tween 20 should be sufficient for most applications. Optimum Tween 20 concentrations will vary to suit specific experiments, but a 0.05% Tween 20 concentration is suitable for most blotting applications.

1. Briefly rinse the membrane with 3 times of Wash Buffer

◈ Primary antibody treatment

1. **Pour off Wash Buffer, dilute the primary antibody in Wash Buffer. The dilution factor should be determined empirically for each antibody.**
2. **Incubate the membrane in primary antibody solution for 1 hour at room temperature on an orbital shaker.**

Note : Incubation times and temperatures may vary and should be optimized for each antibody. The conditions indicated are recommended starting points

3. **Pour off primary antibody solution from membrane, then wash the membrane for 10 min. at room temperature with Wash Buffer**

◈ Secondary antibody Treatment

1. **Pour off Wash Buffer, dilute the secondary antibody in Wash Buffer. The dilution factor should be determined empirically for each antibody.**
2. **Incubate the membrane in secondary antibody solution for 1 hour at room temperature on an orbital shaker.**

Note : Incubation times and temperatures may vary and should be optimized for each antibody. The conditions indicated are recommended starting points.

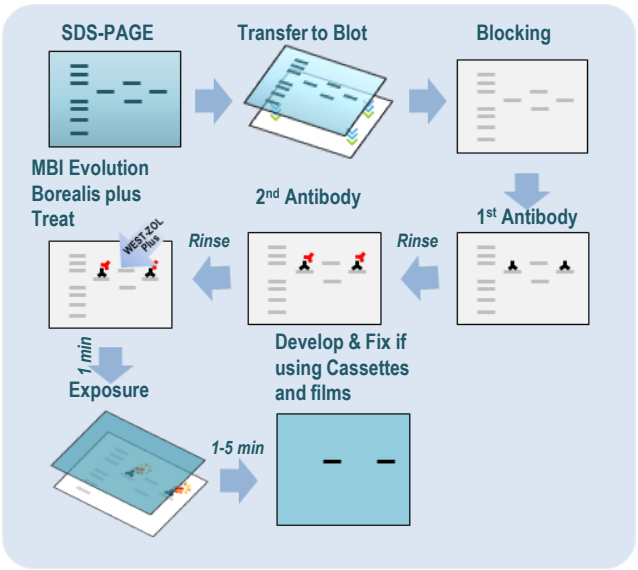
3. **Pour off primary antibody solution from membrane, then wash the membrane for 10 min. at room temperature with Wash Buffer**

MBI Lab Equipment
Toll free 1888 697 2312l
web: www.montreal-biotech.com

◈ Detection and Protocol

1. **Mix an equal volume of Substrate Solution and Enhancer Solution by inversion in a screw cap tube or container to give sufficient to cover the membrane. (Please avoid mixing Substrate solution and Enhancer in bottles while pipetting) s and add the detection reagent to the protein side of the membrane.**
2. **Incubation for 1 to 5 minutes at room temperature without agitation.**
3. **Drain off excess detection reagent by holding the membrane vertically and touching the edge of the membrane with tissue paper.**
   Note : For less background, excess detection reagent on membrane have to be removed.
4. **Wrap membranes if necessary in transparent thin plastic wrap and gently smooth out air bubble. Place the blot(s), protein side up, in the film cassette in dark room or in an imaging system.**
5. **Focus and expose in imaging system or develop film using appropriate developing and fixing reagent**

QUICK GUIDE



TROUBLESHOOTING GUIDE

Problem	Possible Cause	Recommendation
Weak or no signal	Too much HRP in the system depleted the substrate and caused the signal to fade Quickly	Dilute HRP-conjugate at least 10-fold
	Insufficient quantities of antigen or antibody	Increase amount of antibody or antigen
	Inefficient protein transfer	Optimize transfer condition
	Reduction of HRP or substrate activity	To test the activity of the HRP detection system in the darkroom, prepare 1-2mL of the 1:1 mixed MBI Evolution Borealis plus ® Western Blot Detection System in a clea r test tube. With the lights turned off, add 1µL Undiluted HRP-conjugate to the Working Solution. The solution should immediately emit a blue light that will fade over the next several minutes.
High background	Inadequate blocking	Optimize blocking conditions
	Too much HRP in the system	Dilute HRP-conjugate at least 10-fold
	Inappropriate blocking reagent	Try a different blocking reagent
	Inadequate washing	Increase length, number or volume of washes
	Antigen or antibody is too concentrated	Dilute antigen or antibody working solution
white bands Reverse images on film	Antibody and protein concentration	White band generally occur when protein Target is in excess an antibody Concentration is too high.
Spots within the protein bands	Unevenly hydrated membrane	Perform manufacturer's recommendations for hydrating membrane properly
	Bubble between the film and the membrane	Remove all bubbles before exposing blot to film
Speckled back-ground on Film	Aggregate formation in the HRP-conjugate	Filter conjugate through a 0.2 µm filter or centrifuge and use supernatant
Nonspecific bands	Inadequate blocking	Optimize blocking conditions
	Too much HRP in the System	Dilute HRP-conjugate at least 10-fold