WesternMAX™ HRP Chemiluminescent Detection Kits

Code	Description
N222-KIT	WesternMAX™ Chemiluminescent HRP Kit, Anti-Mouse
	Includes: HRP Conjugated Anti-Mouse Antibody, 0.2 mg
	Tween® 20, 20 ml
	Mouse Control IgG, 0.5 ml
	Bovine Serum Albumin (BSA), 10 g
	WesternMAX™ Dilution Buffer Powder, 100 g
	VisiGlo™ HRP Substrate A, 120 ml
	VisiGlo™ HRP Substrate B, 120 ml
N223-KIT	WesternMAX™ Chemiluminescent HRP Kit, Anti-Rabbit
	Includes: HRP Conjugated Anti-Rabbit Antibody, 0.2 mg
	Tween® 20, 20 ml
	Rabbit Control IgG, 0.5 ml
	Bovine Serum Albumin (BSA), 10 g
	WesternMAX™ Dilution Buffer Powder, 100 g
	VisiGlo™ HRP Substrate A, 120 ml
	VisiGlo™ HRP Substrate B, 120 ml

Description:

The design of this kit simplifies the process of Western blotting. The kit contents provide a rapid and sensitive system for the detection of proteins that have been transferred to a membrane and stained with immuno-specific primary and HRP-conjugated secondary antibodies. The basis of detection relies on the use of AMRESCO's VisiGlo™ reagents for HRP detection. This luminol-based, chemiluminescent detection system offers superior advantage in sensitivity compared to chromogenic substrates (i.e., DAB).

Number of Assays:

The supplied materials are sufficient for performing 20 – 30 Western blots equal to 10 cm x 10 cm (100 cm^2 each). The antibody supplied will yield approximately 200 mL of working solution when diluted to the recommended concentration.

Storage / Stability:

This kit is stable for at least one (1) year when stored at +4ºC.

Buffer Preparation:

The WesternMAX™ HRP Detection Kit contains all the necessary components for preparing a Dilution Buffer, Wash Buffer and Blocking buffer. The volume of buffers required will vary depending on the number and size of membrane blots to be treated. Although this kit suggests the use of the supplied Dilution Buffer Powder for preparation of working reagents, Phosphate Buffered Saline (PBS) may be substituted if desired (not supplied). All solutions should be prepared deionized water. If solutions are to be stored for subsequent use, it is recommended they be sterile-filtered and stored at 4ºC. Do not store solutions for greater than one (1) month. The use of preservatives or anti-microbial agents (i.e., Sodium Azide) may interfere with detection of HRP.

Dilution Buffer

- Add 1.2g WesternMAX™ Dilution Buffer Powder to 100 mL deionized water.

Wash Buffer

- Add 100µL of Tween® 20 to 100 mL of Dilution Buffer to make a 0.1% (v/v) Tween® 20 solution

Blocking Buffer (optional)

Add 100 mg Bovine Serum Albumin (BSA) per 10 mL of Wash Buffer to make a 0. 1% (w/v) BSA solution.

VisiGlo™ Development Buffer

- Combine equal volumes of VisiGlo™ Substrate A and VisiGlo™ Substrate B

Secondary Antibody:

Approximately 10 mL of solution is used to cover a 10 cm x 10 cm membrane. To prepare 10 mL of diluted secondary antibody solution (sufficient for 1 – 2 100 cm^2 membranes), add 5 µL of concentrated HRP conjugated secondary (Rabbit or Mouse) antibody to 10 mL of Wash Buffer. This is a recommended starting concentration. Improved results may require some degree of optimization.

Immunoblot Method:

The WesternMAX™ HRP Chemiluminescent Detection Kit can be used for detection of protein antigens on a variety of membranes including PVDF and nitrocellulose. The following method provides a guideline for use. However, it may be necessary, depending upon the antigen, antibody, and specific conditions being used, for further optimization. All steps should be performed at room temperature. The use of a rocking or orbital platform to provide gentle agitation is suggested. In order to reduce background, membranes should not be allowed to dry at any time during the process. Use care when handling membranes. **Always use gloved hands and never allow for skin contact.**

Protocol Overview:

- Prepare sample for assay (Western blot or dot blot).
- Block membrane with Wash Buffer or Blocking Buffer (30 minutes).
- React with Primary antibody.
- React with HRP-conjugated secondary antibody
- React with VisiGlo™ HRP reagent system.
- Visualize proteins by autoradiography.

1. Transfer proteins from an SDS-PAGE gel via electroblotting or other equivalent method. It is recommended the provided IgG matched control be loaded to one lane of the gel for use as a positive control.
2. If the membrane has been allowed to dry following transfer, re-hydrate by submersion in deionized water for 1 – 2 minutes. If PVDF is being used, re-hydrate membrane in a 10% Methanol solution.
3. Block membrane with Wash Buffer or Blocking Buffer for 30 – 60 minutes at room temperature with gentle agitation.
4. Discard blocking solution.
5. Dilute the primary antibody in Wash Buffer or Blocking Buffer. The proper dilution must be determined through trial and error or per the supplier's recommended starting concentration. Add approximately 10 mL or a volume of antibody solution sufficient to cover the surface of the membrane. Incubate membrane at room temperature for 30 – 120 minutes with gentle agitation.
6. Discard primary antibody solution. (Primary antibody solutions may be re-used if stored at 2°C – 8ºC for up to one week).
7. Wash membrane with an ample amount of Wash Buffer for 5 – 10 minutes with gentle agitation and discard. Repeat wash step twice more.
8. Add approximately 10 mL or a volume of secondary antibody solution sufficient to cover the surface of the membrane. Incubate membrane at room temperature for 30 – 60 minutes with gentle agitation.
9. Discard secondary antibody solution.
10. Wash membrane with an ample amount of Wash Buffer for 5 – 10 minutes with gentle agitation and discard. Repeat wash step twice more.
11. For detection, cover membrane with an ample amount of VisiGlo™ Development Buffer. The volume used should be sufficient to just cover surface of membrane. Excess amount of reagent or submersion of membrane may contribute to background.
12. Incubate membrane in VisiGlo™ Development Buffer for 1 – 10 minutes.
13. Gently blot membrane dry with a Kimwipe®, or alternatively, allow excess reagent to run off.
14. Proteins may be visualized via autoradiography following exposure to film.

Additional Notes:

- The above protocol is intended to be used as a general guideline. Deviations from this may be made by experienced users. The buffers and reagents provided have been optimized for use with the given protocol. If conditions, volumes, reaction times, or temperatures are altered, the overall performance of the kit and its reagents may be affected. As a general rule, modifications should be made sequentially one at a time.
- There are several manufacturers of electrophoresis equipment used for Western blotting and gel-running applications. Refer to your owner's manual for an optimized blotting / transfer protocol.
- In most cases, sufficient blocking of the membrane can be achieved with Dilution Buffer with Tween® only. It may be necessary, however, to use Blocking Buffer for improved signal-to-noise ratio.
- It is possible that some primary antibodies may not bind well in the presence of non-ionic detergents such as Tween® 20. For best results, dilute the primary antibody in Blocking Buffer when dealing with an unknown antigen-antibody pair. Alternatively, Dilution Buffer or Phosphate Buffered saline (PBS) containing 1% – 10% goat serum may be used.

Troubleshooting – Blotting:

Problem 1 – Excess Signal or Background

- Decrease film exposure time.
- Decrease HRP conjugate concentration.
- Reduce conjugate incubation time.
- Increase washing or blocking times.
- Load less protein / DNA onto gel.

Problem 2 – No Signal

- Verify transfer by staining protein gel with Coomassie® Blue or DNA gel with Ethidium Bromide.
- Verify protein transfer by staining membrane with Ponceau S or Amido Black.
- Make sure HRP secondary antibody is specific for primary antibody.
- Do not add Sodium Azide to solutions. This will inhibit Peroxidase activity.

Problem 3 – Weak Signal

- Increase film exposure time.
- Increase conjugate concentration.
- Increase conjugate incubation time.
- Load more protein / DNA onto gel.
- Make sure primary antibody has high affinity for target protein. Antibody affinity may change after denaturation of sample with SDS.

References:

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