VisiGlo™ Prime HRP Chemiluminescent Substrate Kit

Code	Description	Size
1B1581-KIT-SAMPLE	**VisiGlo™ Prime HRP Chemiluminescent Substrate Kit**	100 cm^2 (1 mini-blot)
1B1581-KIT	**VisiGlo™ Prime HRP Chemiluminescent Substrate Kit**	1,000 cm^2 (12 mini-blots)

General Information

VisiGlo™ Prime HRP Chemiluminescent Substrate Kit delivers a wide dynamic range of HRP (horse radish peroxidase) detection that is linear over three orders of magnitude, enabling accurate and quantitative comparison of proteins, especially when combined with CCD imaging. Highly sensitive VisiGlo™ Prime is ideal for detection of low abundance proteins, with sensitivity down to attomolar levels. High abundance proteins are detected without exhibiting substrate depletion, an important feature for simultaneous detection of low and high abundance proteins in a single exposure. The chemiluminescent emission from VisiGlo™ Prime is highly stable, lasting hours after substrate incubation to allow time for multiple exposures to obtain optimal blotting images.

- Attomolar sensitivity
- Quantitative – linear range of signal spans 3 orders of magnitude
- Low background for optimal signal intensity
- Sustained signal allows detection hours after substrate incubation
- Compatible with CCD- and x-ray film-based imaging systems

Storage/Stability

Store at room temperature (18 to 26°C).

Product Use Limitations

For research use only. Not for therapeutic or diagnostic use.

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Directions for Use

Materials Supplied

1B1581-KIT-SAMPLE

- 5 mL VisiGlo™ Prime HRP Chemiluminescent Substrate Solution A
- 5 mL VisiGlo™ Chemiluminescent Substrate Solution B

1B1581-KIT-100ML

- 50 mL VisiGlo™ Prime HRP Chemiluminescent Substrate Solution A
- 50 mL VisiGlo™ Chemiluminescent Substrate Solution B

Required Materials Not Supplied

- Protein/lysate containing target
- Electrophoresis apparatus and buffers for SDS-PAGE
- Transfer apparatus and transfer buffer
- Nitrocellulose or PVDF
- Whatman™ blotting paper
- PBS-T or TBS-T wash buffer
- Blocking Buffer
- Primary and secondary antibodies
- CCD-based detection system or x-ray film

Protocol/Procedure

Note: Volumes of buffers for blotting should be 0.3 mL or greater per cm^2 of membrane.

Electrophoresis and Western Blotting

1. Cast an SDS-PAGE gel or use a precast gel of an appropriate percentage to separate the protein of interest by electrophoresis. Any electrophoresis system and buffer are acceptable.
2. Transfer proteins from the gel to a PVDF or nitrocellulose membrane using a wet (tank) or semi-dry transfer method.
3. Incubate the membrane in blocking buffer for 1 hour at room temperature with gentle agitation. The appropriate blocking buffer composition may vary for different proteins and should be optimized as needed.
4. Incubate the membrane in primary antibody that has been diluted into blocking buffer for 1 – 4 hours at room temperature or overnight at 4°C with gentle agitation. Determine optimal primary antibody concentrations empirically. Note that for blots that will be imaged with x-ray film, up to 5X less primary antibody may be required compared to blots imaged with CCD-based system.

5. Wash the blot in excess volumes of TBS-T or PBS-T wash buffer with agitation at room temperature:
    - 1X quick wash
    - 1X 15 minute wash
    - 3X 5 minute washes
6. Incubate the membrane in secondary HRP-conjugated antibody that has been diluted into blocking buffer for 1 hour at room temperature with gentle agitation. Determine the optimal secondary antibody concentration empirically. Note that for blots that will be imaged with x-ray film, up to 5X less primary antibody may be required compared to blots imaged with CCD-based system.
7. Wash the blot 3X 5 minutes in excess volumes of TBS-T or PBS-T wash buffer with agitation at room temperature.

HRP Detection

Note: Do not use metal forceps during detection, as traces of metal may result in high background noise by acting as a catalyst for non-enzymatic substrate oxidation.

1. Prepare a volume of chemiluminescent substrate equal to at least 0.1 mL/cm^2 of membrane by mixing VisiGlo™ Prime HRP Chemiluminescent Substrate Solution A and VisiGlo™ Chemiluminescent Substrate Solution B in a 1:1 ratio. (Working substrate solution is best prepared just before use, although it is stable for several hours at room temperature.)
2. Cover the membrane with working VisiGlo™ Prime substrate solution and allow to react for 2 minutes.
3. Remove excess VisiGlo™ Prime substrate solution and then cover the damp blot with transparent plastic wrap.
4. Proceed with imaging the blot by one of the following methods:
    - CCD-based digital imaging system
    - X-ray film exposure and film development
    - *Recommended initial exposures; 0.5, 2 and 5 minutes.*
    - *Multiple exposures may be taken over the course of several hours. Signal intensity after 1 hour remains at 70% of initial signal intensity. Substantial signal will also be present after 8-10 hours.*

Frequently Asked Questions

Problem	Cause	Solution
High background	Antibody concentration too high	Reduce the primary antibody concentration.
	Too much target	Decrease the amount of target loaded on the gel.
	Insufficient blocking	Try a new blocking buffer composition and/or increase blocking time.
	Insufficient washing	Increase wash buffer volume and increase washing time.
	Overexposure	Decrease exposure time during imaging.
Weak or absent signal	Insufficient target	Increase the amount of target loaded on the gel.
	Insufficient transfer	Verify transfer by staining the gel post-transfer with Coomassie® Blue or by staining the membrane with Poneau S.
	Incorrect secondary antibody used	Verify that the secondary antibody recognizes the primary antibody species.
	Sodium azide present	Do not use sodium azide in solutions used for blotting, as it will inhibit peroxidase activity.
	Insufficient exposure	Increase exposure time during imaging.
	Antibody concentration too low	Increase the concentration of the primary antibody and/or the primary antibody incubation time.
White spots within bands	Air bubbles during transfer	Ensure there are no air bubbles during the transfer process.
Background speckles	Contamination of blotting solutions	Filter blotting solutions to remove contaminants and particulate matter. Use clean, covered containers for blotting steps.

Directions for Use

For Technical Support

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