Directions for Use

VisiGlo™ Select HRP Chemiluminescent Substrate Kit

Code	Description	Size
1B1583-KIT-100ML	**VisiGlo™ Select HRP Chemiluminescent Substrate Kit**	100 mL (12 blots)
1B1583-KIT-SAMPLE	**VisiGlo™ Select HRP Chemiluminescent Substrate Kit**	10 mL (1 blot)

General Information

VWR Life Science AMRESCO's VisiGlo™ Select HRP Chemiluminescent Substrate Kit combines a broad linear dynamic range and attomolar sensitivy to enable quantitation of low-abundance targets labeled with horseradish peroxidase (HRP). The high sensitivity and signal-to-noise ratio allow for target detection with minimal consumption of valuable protein and antibody reagents, thereby lowering experimental costs. Low-intensity signals undetectable with competing chemiluminescent substrates may be revealed as well, because background levels remain low even for lengthy exposure times. The signal generated by substrate incubation is stable for hours and may be captured by conventional x-ray film, CCD and fluorescence-based imaging systems.

- Highest sensitivity – ideal for detecting low-abundance protein
- Quantitative with broad dynamic range
- High signal-to-noise ratio
- Signal lasts hours – enables repeated exposures and imaging delays
- Versatile imaging with x-ray film, CCD – and fluorescence-based imagers

Storage/Stability

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

Product Use Limitations

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

Materials Supplied

Component	1B1583-KIT-100ML	1B1583-KIT-SAMPLE
VisiGlo™ Select HRP Chemiluminescent Substrate Solution A	1B1582-50ML	1B1582-5ML
VisiGlo™ Chemiluminescent Substrate Solution B	1B1580-50ML	1B1580-5ML

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, fluorescence-based 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:
    - 1 X quick wash
    - 1 X 15 minute wash
    - 3 X 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 3 X 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™ Select 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™ Select substrate solution and allow to react for 2 minutes for blots that will be detected using a CCD-based system or x-ray film. If detection will be performed with fluorescent imaging, the incubation time with substrate may be increased to 5 – 10 minutes.

3. Remove excess VisiGlo™ Select 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

- Fluorescence imaging system (The substrate has a blue light excitation range of 430 – 490 nm. The optimal emission range is 500 – 530 nm.)
- 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 60% of initial signal intensity. Substantial signal will also be present after 8-10 hours.
- The blot may be imaged while damp or when dried completely. The fluorescent signal is greater on dried blots, however, the background may also be slightly increased.
- Products of the substrate-HRP oxidation reaction are water-insoluble and will remain adsorbed to the membrane.

Frequently Asked Questions

Problem	Cause	Solution
High Background	1. Antibody concentration too high 2. Too much Target 3. Insufficient blocking 4. Overexposure	1. Reduce the primary antibody concentration 2. Decrease the amount of target loaded on the gel 3. Try a new blocking buffer composition and/or increase blocking time. 4. Decrease exposure time during imaging.
Weak or Absent Signal	1. Insufficient target 2. Insufficent transfer 3. Incorrect secondary antibody used 4. Sodium Azide present 5. Insufficient exposure 6. Antibody concentration too low	1. Increase the amount of target loaded on the gel. 2. Verify the transfer by staining the gel post transfer with Coomassie Blue or by staining the membrane with Poneau S. 3. Verify that the secondary antibody recognizes the primary antibody species. 4. Do no use sodium azide in solutions used for blotting, as it will inhibit peroxidase activity 5. Increase exposure time during imaging 6. 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.

For Technical Support

Toll Free: 1-800-610-2789 (USA & Canada)
Fax: (440) 349-0235
Email: techinquiry@amresco-inc.com

AMRESCO, LLC
A VWR Company

Corporate Headquarters
28600 Fountain Parkway
Solon, Ohio USA 44139-4300

Tel: 440/349-1199
Fax: 440/349-1182
www.amresco-inc.com

VisiGlo™ Select HRP Chemiluminescent Substrate Kit

ZY0604
Rev. 1 12/2015