VWR AMRESCO LIFE SCIENCE

Directions for Use

VisiGlo™ and VisiGlo Plus™

Code	Description	Size
N218-KIT	**VisiGlo™ HRP Chemiluminscent Substrate Kit**	2,400 cm^2 24-10 x 10 cm blots
N218-S-KIT	**VisiGlo™ HRP Chemiluminscent Substrate Kit**	1,200 cm^2 12-10 x 10 cm blots
N218-SAMPLE-KIT	**VisiGlo™ HRP Chemiluminscent Substrate Kit**	100 cm^2 1-10 x 10 cm blot
N219-KIT	**VisiGlo Plus™ HRP Chemiluminscent Substrate Kit**	2,400 cm^2 24-10 x 10 cm blots

General Information

VisiGlo™ and VisiGlo Plus™ are luminol-based chemiluminescent substrate designed for use with horseradish peroxidase (HRP) labeled reporter molecules. In the presence of hydrogen peroxide, HRP converts luminol to an excited intermediate dianion that emits lights upon return to its ground state. The light can be captured on Western blots by film or CCD-based imaging systems or in microwell assays using a luminometer. VisiGlo™ is a reliable, affordable, basic level substrate ideal for routine detection of protein with picogram sensitivity and minimal background. This emission from VisiGlo™ reaches maximum intensity within 5 minutes and is sustained for approximately 1 to 2 hours. VisiGlo Plus™ offers a 20-fold more intense signal than VisiGlo™ and is optimal for the detection of low abundance proteins or the conservation of valuable protein samples.

Storage/Stability

Stable at least one year when stored cold (2 – 8°C). Protect from light.

Product Use Limitations

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

Materials Supplied

VisiGlo™ HRP Components	N218-KIT	N218-S-KIT	N218-SAMPLE-KIT
VisiGlo™ HRP Chemiluminescent Susbrate A	N252-120ML	N252-60ML	N252-5ML
VisiGlo™ HRP Chemiluminescent Susbrate B	N253-120ML	N253-60ML	N253-5ML

VisiGlo Plus™ HRP Components	N219-KIT
VisiGlo Plus™ HRP Susbrate A	N219-40ML
VisiGlo Plus™ HRP Susbrate B	N219-80ML

Required Materials Not Supplied

Protein/lysate containing target
Electrophoresis apparatus and buffers for SDS-PAGE
Transfer apparatus and transfer buffer
Nitrocellulose of PVDF membrane
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

Electrophoresis and Western Blotting

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

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 shaking. Determine optimal primary antibody concentrations empirically.

5. Wash the blot 3 times, 5 minutes each, in excess volumes of TBS with Tween® 20 (TBS-T) or PBS with Tween® 20 (PBS-T) wash buffer with shaking at room temperature.

6. Incubate the membrane in a species appropriate secondary HRP-conjugated antibody that has been diluted into blocking buffer for 1 hour at room temperature with gentle shaking. Determine the optimal secondary antibody concentration empirically.

7. Wash the blot 3 times, 5 minutes each, in excess volumes of TBS-T or PBS-T wash buffer with agitation at room temperature.

8. Proceed to HRP Detection for Membranes.

Electrophoresis and Southern Blotting

Note: Initial exposure of 10 – 15 minutes is recommended for plasmid DNA and 30– 60 minutes is recommended for genomic DNA

1. Cast an agarose gel of an appropriate percentage to separate the DNA of interest by electrophoresis.

2. Transfer DNA from the gel to a positively-charged nylon membrane using a capillary or electroblotting method.

3. Pre-hybridize the membrane for 30 – 60 minutes at the appropriate hybridization temperature.

4. Incubate the membrane 3 – 16 hours at the appropriate hybridization temperature in hybridization solution containing biotinylated probe.

5. Perform stringency washes with SSC or SSPE following standard Southern blotting protocols.

6. Block the membrane 30 – 60 minutes with an appropriate blocking solution.

7. Incubate the membrane in a solution of HRP-Streptavidin diluted in blocking solution. Determine the optimal concentration of HRP-Streptavidin empirically.

8. Transfer the membrane to a clean container and wash 3 times, 5 minutes each, with excess wash solution.

9. Proceed to **HRP Detection for Membranes.**

HRP Detection for Membranes

Note: The working substrate solution is stable for 1 – 2 hours at room temperature or up to 24 hours when stored at 2 – 8°C.

1. Prepare a volume of VisiGlo™ HRP Working Solution equal to at least 0.1 mL per cm^2 of membrane or a volume of VisiGlo Plus™ HRP Working Solution equal to at least 0.05 mL per cm^2 of membrane. (*Working substrate solution is best prepared just before use.)*

    - **VisiGlo™ HRP Working Solution:** Mix equal volumes of VisiGlo™ HRP Chemiluminescent Substrate A and VisiGlo™ HRP Chemiluminescent Substrate B.

    - **VisiGlo Plus™ HRP Working Solution:** Mix one volume of VisiGlo Plus™ HRP Substrate A with two volumes of VisiGlo Plus™ HRP Substrate B.

2. Cover the membrane with VisiGlo™ HRP or VisiGlo Plus™ HRP Working Solution and allow to react for 1 – 2 minutes.

3. Remove excess VisiGlo™ HRP or VisiGlo Plus™ HRP Working 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.

ELISA

Note: The typical light decay of VisiGlo™ HRP and VisiGlo Plus™ HRP in microtiter plates has a $t^{1/2}$ value of 60 minutes. Determine antigen and antibody concentrations empirically.

1. Coat each well in an opaque white microwell plate with 100 µL of antigen diluted in carbonate buffer, pH 9.6. Incubate for 2 hour at room temperature or overnight at 2 – 8°C. Incubate the plate with 300 µL/well blocking solution for 15 – 30 minutes.

2. Incubate the plate with 100 µL/well primary antibody diluted in blocking buffer for 1 hour.

3. Wash the plate 3 times with wash solution.

4. Incubate the plate with 100 µL/well HRP-labeled secondary antibody diluted in blocking solution or other appropriate diluent.

5. Wash the plate 3 times with wash solution.

6. Proceed to **HRP Detection for Microwell Assays**.

HRP Detection for Microwell Assays

Note: Working substrate solution is stable for 1 – 2 hours at room temperature or up to 24 hours when stored at 2 – 8°C.

1. Prepare a volume of chemiluminescent substrate equal to 100 µL/well. (Working substrate solution is best prepared just before use.)

    - **VisiGlo™ HRP Working Solution:** Mix equal volumes of VisiGlo™ HRP Chemiluminescent Substrate A and VisiGlo™ HRP Chemiluminescent Substrate B.

    - **VisiGlo Plus™ HRP Working Solution:** Mix one volume of VisiGlo Plus™ HRP Substrate A with two volumes of VisiGlo Plus™ HRP Substrate B.

2. Add 100 µL VisiGlo™ HRP or VisiGlo Plus™ HRP Working Solution to each well.

3. Measure the signal using a luminometer with 1 second integration time per well at 425 nm. For consistent results, perform the measurement 5 – 45 minutes after substrate addition.

Frequently Asked Questions

Problem	Cause	Solution
High Background	Antibody concentration too high	Reduce 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 washing buffer volume and washing time
	Over exposure	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 the secondary antibody recognizes the primary antibody species
	Sodium Azide present	Do not use sodium azide in solution 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 band	Air bubbles during transfer	Ensure there are no air bubbles during the transfer process
Background speckles	Contamination of blotting solutions	Filter blotting solution to remove contaminants and particulate matter. Use clean, covered containers for blotting steps

Directions for Use

For Technical Support

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