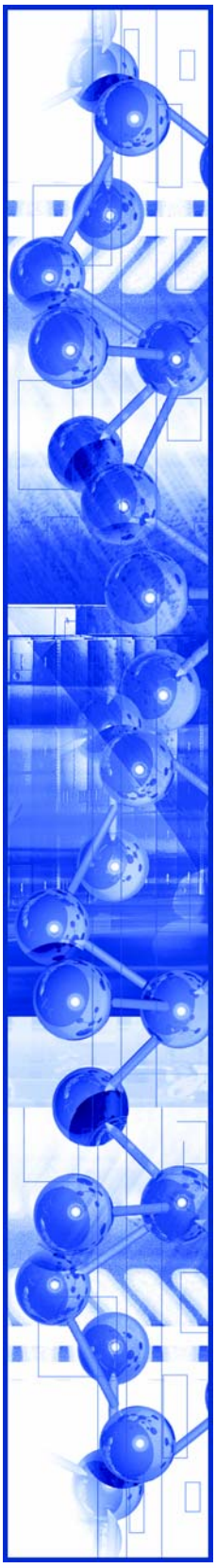

VisiGlo™ / VisiGlo PLUS™ HRP Chemiluminescent Substrates

Chemiluminescent substrates for the detection of Horseradish Peroxidase

Code	Description	Size
N218-KIT	VisiGlo™ HRP Chemiluminescent Substrate Includes: VisiGlo™ HRP Substrate A, 120 ml VisiGlo™ HRP Substrate B, 120 ml *Sufficient material for ~ 2400 cm^2 of membrane.*	240 ml
N218-S-KIT	VisiGlo™ HRP Chemiluminescent Substrate Includes: VisiGlo™ HRP Substrate A, 60 ml VisiGlo™ HRP Substrate B, 60 ml *Sufficient material for ~ 1200 cm^2 of membrane.*	120 ml
N218-SAMPLE-KIT	VisiGlo™ HRP Chemiluminescent Substrate Includes: VisiGlo™ HRP Substrate A, 5 ml VisiGlo™ HRP Substrate B, 5 ml *Sufficient material for ~ 100 cm^2 of membrane.*	10 ml
N219-KIT	VisiGlo PLUS™ HRP Chemiluminescent Substrate Includes: VisiGlo PLUS™ HRP Substrate A, 40 ml VisiGlo PLUS™ HRP Substrate B, 80 ml *Sufficient material for ~ 2400 cm^2 of membrane.*	120 ml

General Information:

VisiGlo™ HRP Substrate is a luminol-based chemiluminescent substrate designed for use with horseradish peroxidase (HRP) labeled reporter molecules. VisiGlo™ HRP provides increased sensitivity over chromogenic substrates in both blotting and microwell assays. Positive reaction sites are rapidly detected with high sensitivity and minimal background. In blotting applications, permanent results are recorded on X-ray film. The use of VisiGlo™ HRP allows for multiple stripping and re-probing of blots. In microwell assays, positive reactions are rapidly detected and read on a luminometer. VisiGlo™ HRP provides a dynamic range that is linear for a longer period of time than other chemiluminescent substrates.

In the presence of hydrogen peroxide, HRP converts luminol to an excited intermediate dianion that emits lights on return to its ground state. This emission from VisiGlo™ HRP reaches maximum intensity within 5 minutes and is sustained for approximately 1 to 2 hours.

VisiGlo PLUS™ HRP offers improved signal intensity – greater than 20-fold more sensitive than VisiGlo™ and other competitive substrates. These products are specifically designed for the detection of low abundant proteins or the conservation of precious protein samples.

Storage/Stability:

VisiGlo™ HRP is supplied as a two component system. Store at 2°C - 8°C. It is stable for a minimum of 1 year from date of receipt when stored at 2°C - 8°C.

Application Disclaimer

For Research Use Only.
Not for Therapeutic or Diagnostic Use.

Applications:

VisiGlo™ / VisiGlo PLUS™ HRP Substrates can be used in both microwell and blotting applications such as ELISA, Western Blotting, Southern Blotting, Dot Blotting, plaque and colony hybridizations.

Reagents Included:

VisiGlo™ HRP Chemiluminescent Substrate Kit:

- VisiGlo™ HRP Chemiluminescent Substrate A
- VisiGlo™ HRP Chemiluminescent Substrate B

or

VisiGlo PLUS™ HRP Chemiluminescent Substrate Kit:

- VisiGlo PLUS™ HRP Chemiluminescent Substrate A
- VisiGlo PLUS™ HRP Chemiluminescent Substrate B

Notes:

- Warm all solutions to room temperature prior to use.
- Solutions do not need to be protected from light.
- VisiGlo™ HRP can be used with nitrocellulose, nylon and PVDF membranes.
- Milk or casein-based blocking solutions are recommended for use with VisiGlo™ HRP. BSA or serum-based blocking agents may cause elevated background.
- All steps are performed at room temperature unless otherwise noted.
- Working solution is stable for up to 1 hour at room temperature or up to 24 hours when stored at 2 °C – 8 °C.

Protocol:

Working Solution Preparation:

VisiGlo™ HRP Chemiluminescent Substrate

- Prepare 1 ml per 10 cm^2 membrane of Working Solution
- Mix equal volumes of Substrate A and Substrate B
- Solution is stable for up to 1 hour at room temperature or up to 24 hours when stored at 2 °C – 8 °C.

VisiGlo PLUS™ HRP Chemiluminescent Substrate

- Prepare 0.5 ml per 10 cm^2 membrane of Working Solution
- Mix 1 volume of Substrate A with 2 volumes of Substrate B.
- Solution is stable for up to 1 hour at room temperature or up to 24 hours when stored at 2 °C – 8 °C.

Western Blotting Protocol:

1. Perform gel electrophoresis and transfer using standard procedures.
2. Block the membrane with an appropriate blocking solution, for 1 hour at room temperature or overnight at 2°C - 8°C. (See AMRESCO's selection of powdered blocking agents.)
3. Incubate membrane with primary antibody or serum sample, diluted in blocking solution, for 1 hour.
4. Wash membrane with appropriate wash solution, 3 times for 5 minutes each. (TBS with Tween® 20, Powder Blend –M235-12.5G-5PK is recommended.)
5. Incubate with HRP conjugate, diluted in blocking solution, for 1 hour. The concentration of HRP conjugate must be determined experimentally.
6. Wash the membrane 3 times for 5 minutes each.
7. Use approximately 1 ml per 10 cm^2 membrane for VisiGlo™ HRP substrate (N218) Working solution **or** 0.5 ml per 10 cm^2 membrane for VisiGlo PLUS™ HRP (N219) Working solution. Incubate membrane for 1 minute in the substrate working solution.
8. Remove membrane from the substrate solution and touch the corner to a piece of filter paper to remove excess solution. Place membrane between plastic sheets or in a hybridization bag.
9. Expose membrane to X-ray film for 1 to 10 minutes. The signal obtained from the first exposure will allow the researcher to determine an exposure time for optimal signal.

Southern Blotting Protocol:

1. Perform gel electrophoresis and transfer using standard procedures.
2. Pre-hybridize membrane for 30 minutes to 1 hour at the appropriate hybridization temperature.
3. Add biotinylated probe to hybridization solution and hybridize 3 – 16 hours at the appropriate hybridization temperature.
4. Following hybridization, perform stringency washes with SSC (0804-4L) or SSPE (0810-4L) following standard protocols.
5. Block membrane for 30 minutes to 1 hour with an appropriate blocking solution. (See AMRESCO's assortment of powdered blocking agents.)
6. Incubate with HRP-Streptavidin, diluted in blocking solution, for 20 to 30 minutes. The optimal concentration of HRP-Streptavidin must be determined experimentally.
7. Transfer membrane to a clean container and wash with an appropriate wash solution. Wash the membrane 3 times for 5 minutes each.
8. Use approximately 1 ml per 10 cm^2 membrane for VisiGlo™ HRP substrate (N218) Working solution **or** 0.5 ml per 10 cm^2 membrane for VisiGlo PLUS™ HRP (N219) Working solution. Incubate membrane for 1 minute in the substrate working solution.

9. Remove membrane from substrate solution and touch the corner to a piece of filter paper. Place membrane between plastic sheets or in a hybridization bag.

10. Expose membrane to X-ray film. An initial exposure of 10 to 15 minutes is recommended for plasmid DNA and 30 to 60 minutes is recommended for genomic DNA. The signal obtained from the first exposure will allow the researcher to determine an exposure time for optimal signal.

Elisa Protocol:

All steps are at room temperature unless otherwise noted.

➥ **Note:** The typical light decay of VisiGlo™ in microtiter plates has a $t_{1/2}$ value of 60 minutes.

1. Coat each well in an opaque white microwell plate with 100 µL antigen, diluted in carbonate buffer (pH 9.6), for 2 hours at room temperature or overnight at 2 – 8°C. Optimal antigen dilution must be determined experimentally.
2. Block plate for 15 - 30 minutes using 300 µL/well of appropriate blocking solution.
3. Incubate plate for 1 hour with 100 µL/well primary antibody diluted in blocking solution. Optimal antibody dilution must be determined experimentally.
4. Wash plate 3 times with wash solution.
5. Incubate plate for 30 minutes to 1 hour with 100 µL/well HRP-labeled secondary antibody diluted in blocking solution or other appropriate diluent. Optimal antibody dilution must be determined experimentally.
6. Wash plate 3 times.
7. Prepare VisiGlo™ Chemiluminescent Substrate by mixing equal volumes of Substrate A and Substrate B.
8. Add 100 µLl VisiGlo™ working solution to each well.
9. Read in a luminometer with 1 second integration time per well. VisiGlo™ provides consistent results when read 5 - 45 minutes after addition of substrate.

Related Products

Code	Product
Reversible Protein Gel Stain	
M277-KIT	ZiP™ Reversible Protein Detection Kit *Contains sufficient reagents to stain 25-50 mini-gels.*
Western Blotting Transfer Buffer	
M279-500ML	NEXT GEL™ Transfer Buffer, 10X
Protein Membrane Stain	
M282-1L	ProAct™ Membrane Stain
Blocking Buffers	
M235-12.5G-5PK	TBS with Tween® 20 Includes: 5 pouches *Each pouch makes 1 L of a 1X solution.*
M231-22G-5PK	TBS with BSA Includes: 5 pouches *Each pouch makes 1 L of a 1X solution.*
M230-42G-5PK	TBS with Non-Fat Milk Powder Includes: 5 pouches *Each pouch makes 1 L of a 1X solution.*
M245-10.4G-5PK	PBS with 0.05% Tween® 20, pH 7.4 Includes: 5 pouches *Each pouch makes 1 L of a 1X solution.*
M233-19.8G-5PK	PBS with BSA Includes: 5 pouches *Each pouch makes 1 L of a 1X solution.*
M232-39.8G-5PK	PBS with Non-Fat Milk Powder Includes: 5 pouches *Each pouch makes 1 L of a 1X solution.*
Wash Buffers & Detergents	
K875-500ML	PBS with Tween® 20 Buffer, 20X
K873-500ML	TBS with Tween® 20 Buffer, 20X
M228-10ML-5PK	Tween® 20, 10% Solution, Peroxide-free 10 ml ampoules
Hybridization Buffers	
0804-4L	SSC Buffer, 20X Liquid Concentrate
0810-4L	SSPE, 20X Liquid Concentrate
Enzyme Conjugate	
0343-10KU 0343-25KU	Horseradish Peroxidase (HRP) ≥ 250 Purpurogallin Units/mg
0417-25KU 0417-100KU	Horseradish Peroxidase (HRP) ≥ 100 Purpurogallin Units/mg

Troubleshooting:

Blotting Procedures

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.

No Signal

- Verify transfer by staining protein gel with Coomassie® Blue or DNA gel with Ethidium Bromide.
- Verify protein transfer by staining membrane with ProAct™ Membrane Stain (M282-1L), Ponceau S or Amido Black.
- Make sure HRP secondary antibody is specific for primary antibody.
- Do not add Sodium Azide in solutions. This will inhibit Peroxidase activity.

Weak Signal

- Increase film exposure time.
- Increase secondary antibody concentration.
- Increase secondary antibody 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.

ELISA

Excess Signal or Background

- Decrease HRP conjugate concentration.
- Reduce conjugate incubation times.
- Increase washing or blocking times.
- Decrease the amount of protein coated to
- plate.
- High signal wells can contaminate adjacent wells. Remove substrate from suspect well and place in another well to get a more accurate reading.

No signal

- Make sure HRP secondary antibody is specific for the primary antibody.
- Do not add sodium azide to solutions, this will inhibit peroxidase activity.
- Verify the luminometer is working correctly

Weak signal

- Increase conjugate concentration.
- Increase conjugate incubation time.
- Increase the amount of protein coated to the plate.
- Make sure primary antibody has high affinity for target protein.

References:

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