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Product Information

VisiGlo™ / VisiGlo PLUS™
AP Chemiluminescent Substrates

Chemiluminescent substrates for the detection of alkaline phosphatase

Code	Description	Size
N216-200ML	VisiGlo™ AP Chemiluminescent Substrate, 1X solution *Contains sufficient reagents to perform forty 10 cm x 10 cm blots.*	200 ml
N216-100ML	VisiGlo™ AP Chemiluminescent Substrate, 1X solution *Contains sufficient reagents to perform twenty 10 cm x 10 cm blots.*	100 ml
N216-50ML	VisiGlo™ AP Chemiluminescent Substrate, 1X solution *Contains sufficient reagents to perform ten 10 cm x 10 cm blots.*	50 ml
N216-SMPL-15ML	VisiGlo™ AP Chemiluminescent Substrate Sample, 1X solution *Contains sufficient reagents to perform three 10 cm x 10 cm blots.*	15 ml
N217-100ML	VisiGlo PLUS™ AP Chemiluminescent Substrate, 1X solution *Contains sufficient reagents to perform twenty 10 cm x 10 cm blots.*	100 ml

General Information:

VisiGlo™ Alkaline Phosphatase (AP) Substrate is a sensitive chemiluminescent substrate for the detection of phosphatase labeled reporter molecules on Western blots and in ELISA assays. The high intensity and low background of the dioxetane-based chemiluminescent substrate in VisiGlo™ improves signal strength relative to traditional chromogenic and chemiluminescent substrates. Results can be recorded on X-ray film or digitized with a chemiluminescent imager to provide a permanent record. A luminometer should be used for ELISA.

VisiGlo™ AP Substrate is provided as a stable one-component solution. The product provides rapid and accurate identification of proteins in routine assays.

VisiGlo PLUS™ AP Substrate is also provided as a stable one-component solution. This product offers rapid and accurate identification of proteins that are of low abundance and potentially limited availability. The increased sensitivity may reduce the amount of target required for Western blots and ELISAs.

Storage/Stability:

Store this product at 2°C - 8°C. VisiGlo™ solution should remain stored in its original container and protected from light.

The product is stable for a minimum of 2 years from date of receipt when stored under proper conditions.

Application Disclaimer

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

Reagents Included:

VisiGlo™ AP Chemiluminescent Substrate or
VisiGlo PLUS™ AP Chemiluminescent Substrate

Provided as a 1X working solution

➥ **Notes:**

- The VisiGlo™ working solution should be protected from light and warmed to room temperature prior to use.
- VisiGlo™ can be used with either nitrocellulose or PVDF membranes. For best results, nitrocellulose is recommended.
- Insufficient washing of membranes or contamination of substrate with AP will result in non-specific background because of the high sensitivity of VisiGlo™.
- Each lot of AP secondary antibody should be titrated to determine optimal dilution.
- Do not allow VisiGlo™ to contact the film as it will result in the appearance of dark spots.
- Determine optimal exposure periods for X-ray film by performing an initial 1 minute and a 10 minute exposure. Because of its high light intensity, images may be captured over the course of many different intervals. Light emission will continue over the course of 5 to 7 days.

Secondary Antibody Optimization Prior to Detection

- The optimal dilution of each lot of secondary antibody should be determined prior to use since slight differences in activity can produce major differences in background.
- Recommended secondary antibody dilution range should include dilutions from 1/10,000 to 1/100,000 of a 0.1mg/mL stock.

Required reagents not included in kit:

Western Blot Detection:

- Primary antibody
- AP-labeled secondary antibody
- Nitrocellulose or PVDF membrane
- X-ray film (double emulsion) or CCD Imager
- Platform shaker or rocker
- Developing chemicals / equipment
- Incubation trays or tubes
- 0.2M Tris, pH 9.5 – 9.7

Applications:

VisiGlo™ / VisiGlo PLUS™ AP Substrates is optimized for Western blotting and dot blotting applications. It is also suitable for use in microplate assays such as ELISA. The following procedure is recommended for Western blot detection.

Western Blot Protocol Overview

(Total blotting time = 4 hours)

1. Polyacrylamide gel electrophoresis
2. Immobilize protein on membrane
3. Block membrane (1 hour or overnight)
4. Incubate with primary antibody (1 hour)
5. Wash membrane (3 x 5 minutes, 1 x 10 minutes)
6. Incubate with conjugate (30 minutes to 1 hour)
7. Wash membrane (3 x 5 minutes, 1 x 10 minutes)
8. Rinse with assay buffer (2 x 2 minutes)
9. Incubate with VisiGlo™ Substrate (1 minute)
10. Expose to film (10 seconds to 10 minutes)

Protocol:

1. Block the membrane by immersing in block solution (see AMRESCO's assortment of powdered blocking agents) using a minimum of 0.2 ml/cm^2 of membrane. Block at room temperature for 1 hour with gentle rocking or shaking, or stationary at 2°C - 8°C overnight.

   Example *– For a 10 cm x 10 cm blot, use 20 ml of block. Make sure to use a container of proper size that allows the block solution to float freely over the membrane.*

2. Incubate membrane with primary antibody or serum sample for at least 1 hour. This antibody should be added directly to the Block Solution that was used for blocking (see Step 1 above).

   ➥ *Test serial dilutions through a dot blot to determine the optimal working dilution. Alternatively, use the recommended concentration determined by the primary antibody supplier.*

3. Wash the membrane in a generous amount of 1X Wash Solution (at least 25 ml for a 100 cm^2 membrane). Wash membrane 3 times for 5 minutes each, followed by an additional 10 minute wash.

   1X TBS-Tween® may be used (M235-12.5G-5PK).

4. Dilute appropriate secondary antibody 1:10,000 – 1:100,000 (of a 0.1 mg/ml stock) in freshly prepared secondary antibody diluent using a minimum of 0.2 mL/cm^2 of membrane.

   Example *– 2 µL secondary antibody + 20 ml diluent. Suggested diluents include TBS-Tween® (M235-12.5G-5PK). The optimal dilution may vary for different lots of secondary antibody and. Titrate the secondary antibody to determine the optimal working dilution.*

5. Incubate blot with diluted secondary antibody for 1 hour at room temperature.

   ➥During the secondary antibody incubation step, bring *VisiGlo™ or VisiGlo PLUS™ AP* to room temperature. Protect from light.

6. After the secondary antibody incubation, wash as described in Step 3.

7. Pour off the remaining wash buffer from the blot and place the membrane on a sheet protector or a dry tray.

8. Rinse membrane twice for 2 minutes with 0.2M Tris, pH 9.5 – 9.7.

9. Gently pipette room temperature VisiGlo™ or VisiGlo PLUS™ AP over the entire membrane. Use 0.05 ml of substrate solution per cm^2 of membrane. Incubate without rocking for 1 minute.

   Example *– For a 10 cm x 10 cm blot, use 5 ml of VisiGlo™ or VisiGlo PLUS™ AP. The surface tension of the substrate solution will keep it on the surface of the membrane.*

10. Lift the membrane with forceps and blot the excess substrate onto a piece of filter paper. Seal the membrane in clear plastic and expose to X-ray film for 10 seconds to 1 minute.Adjust exposure time as needed.

   ➥ *Excessive substrate on the blot will contribute to background.*

   Caution: *The blotted surface of the membrane must face the film. Do not allow the film to get wet, nor move during exposure.*

   Optimal exposure time should be determined by the signal to noise ratio and the amount of secondary antibody used. When using greater amounts of secondary antibody, 10 seconds may provide acceptable results.

11. Optional – *Chemiluminescent Imager Detection.* Incubate the blot for twice the time typically used for film. If the imager provides stacking capabilities, capture exposures at 5 minutes intervals for 1 hour to maximize the signal. The optimal exposure can be chosen.

   Follow the manufacturer's recommendations regarding the set-up and operation of the imager.

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
Enzyme Conjugate	
E552-1000UNITS	Alkaline Phosphatase (Alk Phos)

Troubleshooting:

No Signal

- Inactive alkaline phosphatase
  Verify enzyme activity by mixing 10 µL of diluted secondary antibody with 1 ml of substrate. (The substrate should glow in the dark.)
- No binding of secondary antibody to the primary antibody
  Confirm correct specificity of the secondary antibody for the primary antibody (i.e., no AP-anti-rabbit with a mouse primary antibody).
- No transfer of target to membrane
  Use a protein stain on unblocked membrane to verify attachment of target protein or use a pre-stained protein marker to monitor transfer.
- Detection of non-blotted side of membrane
  Ensure correct orientation of the membrane during the assay and film exposure.
- Missed step in procedure
  Review procedure to ensure all steps were followed.

Weak Signal

- Insufficient amount of antibody
  Optimize antibody concentrations. Affinity of the primary antibody may change after proteins are denatured through SDS-PAGE.
- Insufficient protein loaded or transferred
  Increase the amount of protein loaded onto the gel.
- Insufficient incubation of primary antibody
  Increase the incubation times for weak primary antibodies.
- Insufficient exposure time
  Increase the time of exposure to film.
- Excessive washing beyond recommended procedure
  Follow the procedure as written.

Excessive Signal, Nonspecific Bands or General Background

- Overexposure of film to signal
  Expose the membrane to film for a shorter period of time.
- Insufficient blocking or washing
  Increase blocking and washing time or increase number of washes. Try a different blocking agent.
- Excessive antibody used for detection
  Optimize secondary antibody concentration. Reduce antibody concentrations; optimal secondary antibody dilution should be 1:10,000 – 1:100,000 of a 0.1 mg/ml stock – OR – Decrease the amount of primary antibody.
- Excessive protein loaded on the gel
  Decrease the amount of protein loaded onto the gel.

Poorly Defined or "Fuzzy" Bands or Dots

- Poor transfer of protein to membrane
  Follow manufacturer's recommended procedure or contact the manufacturer for additional support regarding the blotting apparatus.
- Excessive substrate
  Remove excess substrate before exposure of the membrane to film.
- Ghost images from shifted position of film during development
  Avoid movement of film over membrane during exposure period.
- Inadequate handling of membranes
  Certain membranes require special handling. Check with the membrane vendor for correct procedures.

References:

1. Kricka, L. J. (1991). Chemiluminescent and Bioluminescent Techniques. *Clin. Chem.* 37(9): 1472 – 1481.
2. Knect, D. A. and R. L. Dimond (1984). Visualization of Antigenic Proteins on Western Blots. *Anal. Biochem.* 126: 180 – 184.
3. Blake, M. S., et al (1984). A Rapid, Sensitive Method for Detection of Alkaline Phosphatase Secondary antibody Antibody on Western Blots. *Anal. Biochem.* 136: 175 – 178.
4. Isacsson, V. and G. Wettermark (1974). Chemiluminescence in Analytical Chemistry. *Anal. Chim. Acta.* 68: 339- 362.
5. Towbin, H., T. Staehelin and J. Gordon (1979). Electrophoretic Transfer of Proteins from Polyacrylamide Gels to Nitrocellulose Sheets: Procedure and Some Applications. *Proc. Natl. Acad. Sci. USA.* 76: 4350 – 4354.
6. Bittner, M., P. Kupferer and C. F. Morris (1980). Electrophoretic Transfer of Proteins and Nucleic Acids from Slab Gels to Diazonbenzyloxymethyl Cellulose or Nitrocellulose Sheets. *Anal. Biochem.* 102: 459 – 471.
7. Burnette, W. N. (1980). "Western Blotting": Electrophoretic Transfer of Proteins from Sodium Dodecyl Sulfate-Polyacrylamide Gels to Unmodified Nitrocellulose or Nitrocellulose Sheets. *Anal. Biochem.* 112: 195 – 203.
8. Reinhart, M. P. and D. Malamud (1982). Protein Transfer from Isoelectric Focusing Gels: The Native Blot. *Anal. Biochem.* 123: 229 – 235.
9. Gooderham, K. (1983). *Protein Blotting. In* J. Walker and W. Gaastra (eds.), Techniques in Molecular Biology. Croom Helm Ltd. Publishers, London.
10. Southern, E. M. (1975). Detection of Specific Sequences among DNA Fragments Separated by Gel Electrophoresis. *J. Mol. Biol.* 98: 503 – 517.
11. Kaufmann, Ewing and Shaper (1987). The Erasable Western Blot. *Anal. Biochem.* 161: 89 – 95.

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