VISUAL PROTEIN

VisPRO™ 5 minutes Protein Stain Kit

VisPRO™ 5 minutes Protein Stain Kit is a reverse staining method

The imidazole-zinc reverse stain, utilizing imidazole and zinc ions for protein visualization on electrophoretic gels, was originally introduced in the 1990s [1]. This method is based on the selective precipitation of the imidazole-zinc complex in the gel, except in zones where proteins or other macromolecules are present. Similar to negative film, this method only stains the gel rather than the target proteins. The VisPRO™ 5 minutes Protein Stain Kit is a modified version of the imidazole-zinc reverse stain, designed for convenience and superior performance.

Positive Staining vs. Reverse Staining

Most protein gel staining methods apply the principles of positive staining. To obtain an ideal result, the dye molecules must be completely absorbed by target proteins, and that may require a prolonged incubation of many hours, even up to one day. Furthermore, a tedious de-staining procedure may be required to remove the undesirable staining from the background.

Unlike conventional positive staining methods, reverse staining can be completed in a short time because the target of staining is gel background rather than the proteins. Therefore, reverse staining methods allow researchers to obtain results promptly (Figure 1).

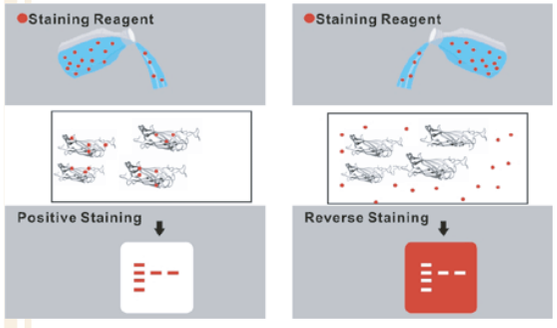


Figure 1. Positive staining vs. Reverse staining

Highlights

Lightning Speed : a 5-minute staining process

Excellent Sensitivity : detect protein levels accurate to 1 ng

High Compatibility with Downstream Applications : can be re-stainedby other staining methods

Safe and Easy to Use : highly stable, non-toxic, no expensive or complex devices are needed

Lightning Speed

To avoid diffusion of proteins, most positive staining methods require an acid fixation prior to the staining procedure. However, the VisPRO™ 5 minutes Protein Stain Kit can complete the staining in 5 min without a pre-staining acid fixation, thus the time for the process can be greatly shortened.

Excellent Sensitivity

Numerous tests have demonstrated that VisPRO™ 5 minutes Protein Stain Kit delivers better staining sensitivity than SYPRO Ruby™ stain, silver stain, and Coomassie Brilliant Blue stain (CBR stain) (Figure 3). This kit offers a dynamic staining range from micrograms to nanograms (even as little as 1 ng of protein can be detected). Unlike other staining methods requiring the interaction between dye molecules and proteins to generate a staining preference between protein species, the reverse staining method provided by the VisPRO™ 5 minutes Protein Stain Kit allows observations of all proteins. However, more protein spots can be found on the two-dimensional electrophoresis (2-DE) gels developed by the VisPRO™ 5 minutes Protein Stain Kit than other high sensitivity protein staining methods, such as silver stain (Figure 4).

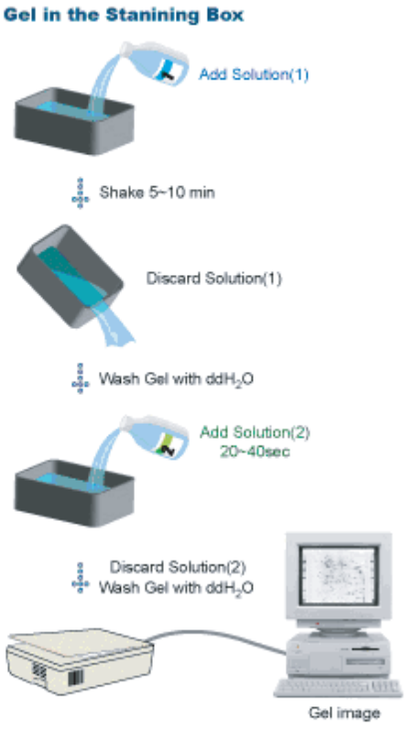


Figure 2.Protocol for VisPRO™ 5 minutes Protein Stain Kit

High Compatibility with Downstream Applications

Gels stained with the VisPRO™ 5 minutes Protein Stain kit can be re-stained by other protein staining methods. In addition, the VisPRO™ 5 minutes Protein Stain is also compatible with many other applications used currently in life science research (Figure 5). Since this method require no acidic fixation, proteins in reverse stained gels can be eluted or electroblotted onto PVDF or nitrocellulose membranes. The VisPRO™ 5 minutes Protein Stain has also been proven to be fully compatible with most mass spectrometers, such as MALDI-TOF (Figure 6). The unfixed proteins are more susceptible to proteolysis and thus easily evaluated by mass spectrometry.

Safe and Easy to Use

The VisPRO™ 5 minutes Protein Stain Kit contains two solutions, Solution I and II, which are ready to use upon opening. No dilution or preparation of other solution is required. It is highly stable, non-toxic, and can be stored at room temperature, not biohazardous to operators or the environment.

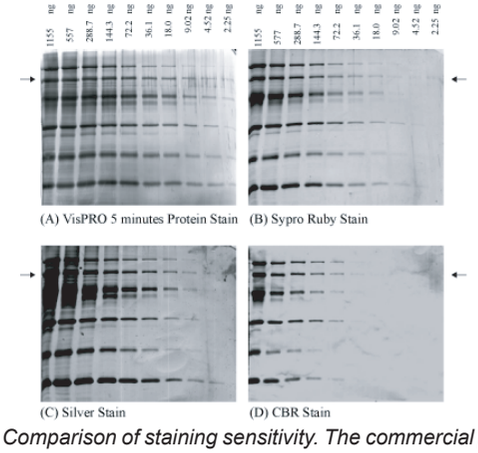


Figure 3. Comparison of staining sensitivity. The commercial protein markers (Amersham, GE healthcare) were used for evaluation. Numbers shown above the gels indicate the actual amount of bovine serum albumin protein (indicated by arrow).

VisPRO™ 5 minutes Protein Stain Kit

Procedures for using:

1. Pour Solution I into a dark staining box (BS1001) containing the electrophoretic gel.
2. Incubate and shake for 5-10 minutes, then briefly rinse with distilled water.
3. Add Solution II and the gel will be developed in 20-40 seconds.
4. Observe the electrophoretic result against a dark background. The GL1001 backlit light plate is suggested to deliver a better visualization.

Documenting the image for the reverse stained gel is straightforward. Simply scan the reverse stained gel on an optical light flatbed scanner with a transparency unit or photograph it by a CCD camera against a dark background (Figure 2). There is no need to purchase expensive imaging acquisition devices such as laser gel scanners.

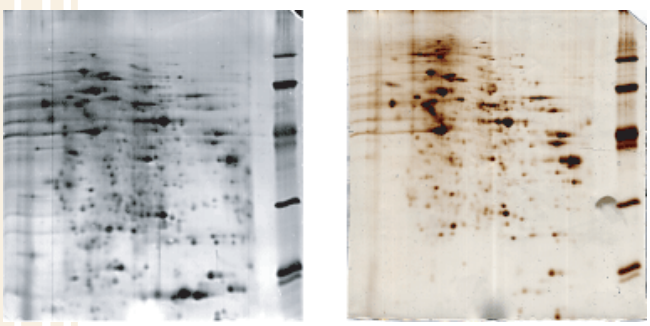


Figure 4. Comparison of staining results for 2-DE gels. (A) VisPRO™ 5 minutes Protein Stain Kit and (B) silver stain.

Recommended application for the VisPRO™ 5 minutes Protein Stain Kit in current proteomics

The recommended application of the VisPRO™ 5 minute Protein Stain Kit is shown in Figure 7. The 2-DE results can be previewed on a backlit light plate. If the results of 2-DE are not ideal, the 2-DE procedure can be repeated immediately. Contrarily, the operator can decide to:

(1) Document the VisPRO™ stained gels directly by using an optical scanner with a transparency unit
(2) After staining gels with SYPRO Ruby™ stain. Researchers can restain the SYPRO Ruby™ stained gels with the VisPRO™ 5 minute-Protein Stain Kit to pick up the protein spots under visible light to prevent UV damage.
(3) After the DIGE stain, the gels can also be re-stained by VisPRO™5 minute Protein Stain Kit for a clearer vision to pick up the protein spots.

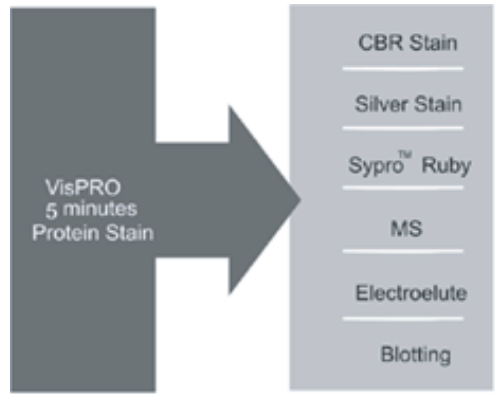


Figure 5.Compatibility of the VisPRO™ 5 minutes Protein Stain Kit with other applications.

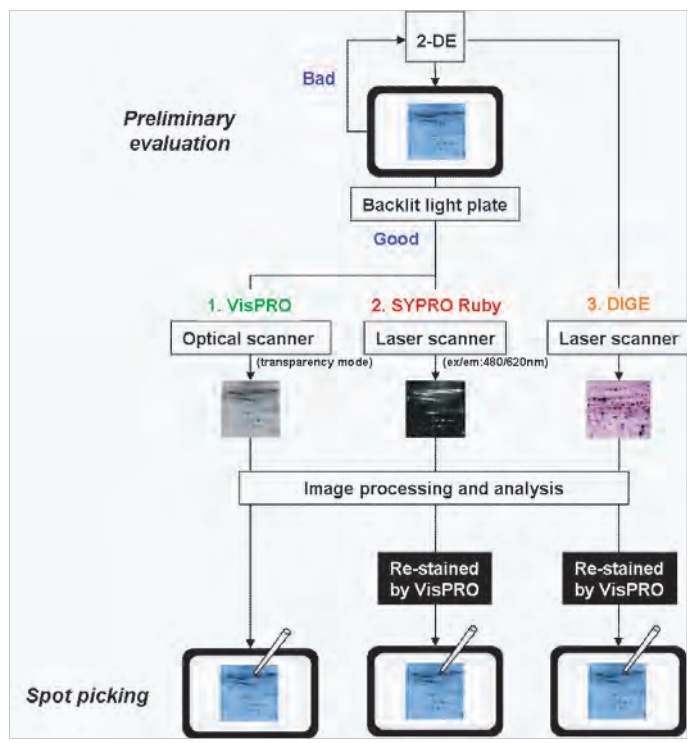


Figure 7. Suggested applications of the VisPRO™ 5 minutes Protein Stain Kit in current proteomic research.

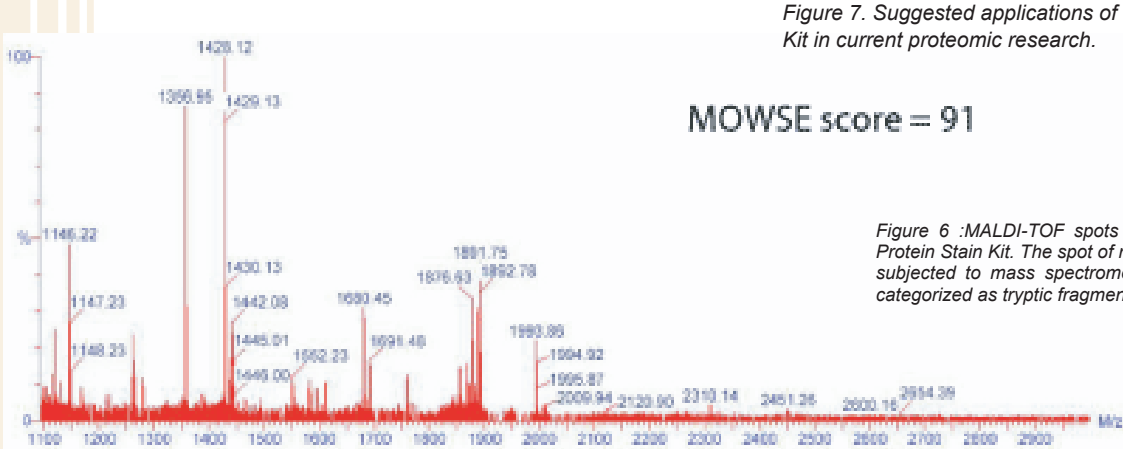


Figure 6 :MALDI-TOF spots visualized with the VisPRO™ 5 minutes Protein Stain Kit. The spot of rabbit phosphorylase b (10 ng) was cut and subjected to mass spectrometry. Twenty-two out of fifty signals were categorized as tryptic fragments of phosphorylase b.

VISUAL PROTEIN

VisPRO™ 5 minutes Protein Stain Kit

Comparison of staining methods

Methods	VisPRO™	SYPRO Ruby™	Silver Stain	CBR Stain
Preparation of solutions	0 min	5 min	20 min	0 min
Fixation	0 min	1 hr	1hr~o/n	0 min
Staining procedure	5~10 min	30 min~o/n	~2 hr	30 min~o/n
De-staining procedure	0 min	1 hour	0 min	2 hr~o/n
Total Time	5 ~15 min	2.5~o/n	3.3~o/n	2.5 hr~o/n
Irritants or toxic chemicals	None	Acetic acid, Methanol	Acetic acid, Silver nitrate, Glutaraldehyde	Acetic acid, Methanol
Sensitivity	<1 ng	1 ng	<1 ng	50 ng
Quantitative range	1-200 ng	1-1000 ng	1-80ng	50-500 ng
Compatibility to downstream applications (e.g. Western blot)	Yes	No	No	No

(o/n: over night; CBR stain: Coomassie Brilliant Blue stain)

Related reference

Reverse staining of sodium dodecyl sulfate polyacrylamide gels by imidazole-zinc salts: sensitive detection of unmodified proteins.

Fernandez-Patron C., Castellanos-Serra L., Rodriguez P.
Biotechniques 1992 Apr; 12(4):564-73.

Use of backlit light plate to enhance visualization of imidazole-zinc reverse stained gels

Ching-Yu Lin, Hui-Ming Huang, and Han-Min Chen
Biotechniques 2006 Nov; 41:560-564

Accessories for VisPRO™ 5 minutes Protein Stain Kit

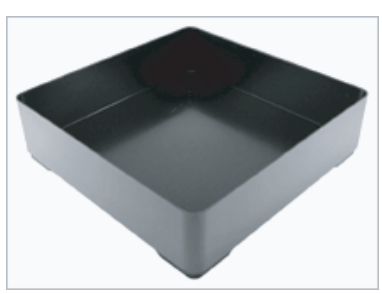
The location of proteins on stained gels developed by the VisPRO™ 5 minutes Protein Stain Kit can be easily visualized against a dark background with naked eye. The Visual Protein Gel Lighting Plate and Black Staining Box are excellent options to achieve a better visual effect.

Gel Lighting Plate

Place an image for the Gel Lighting Plate

The Gel Lighting Plate can enhance the contrast between reverse-stained area of the gel and the unstained proteins to provide a clearer vision when you pick up the protein spots of interest.

Black Staining Box

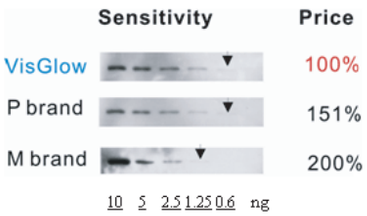


Place an image for the Black Staining Box

The Black Staining Box can help you to observe the staining status and determine when to stop the reaction.

VisGlow™ Chemiluminescent Substrate, HRP

The VisGlow™ Chemiluminescent Substrate, Horseradish Peroxidase (HRP) system promotes a superior chemiluminescent detection of immobilized proteins (e.g. Western blot). Images can be acquired by exposure to X-ray films or cool CCD imaging acquisition systems. The VisGlow™ Chemiluminescent Substrate, HRP system was provided with high quality, and the figure indicates its high sensitivity compared to two other leading brands.



Highlights

Lightning Speed : a 5-minute staining process

High sensitivity : detect target proteins to pg levels

Antibody saving : low antibody dilution delivers high quality result

Low crosstalk : reduce background noises and non-specific signals from irrelevant proteins

Cost-saving : excellent quality with reasonable price

Characteristics of VisGlow™ Chemiluminescent Substrate, HRP

Duration	>1 hours
Detection Method	X-ray film or imaging acquisition system
Typical Antibody Dilution*	Primary: 1:1,000–1:20,000 Secondary: 1:20,000–1:200,000
Shelf life	6 months
Recommended Initial Exposure Time	30 seconds (to films)
Recommended Optimal Exposure Time	5 minutes
Storage Conditions	2–8 °C

VisGlow™ *plus* Chemiluminescent Substrate,HRP

The VisGlow™ plus Chemiluminescent Substrate, HRP system is an enhanced chemiluminescent substrate with advanced efficacy and sensitivity for chemiluminescent detection. The VisGlow™ plus is at least 8-fold more sensitive than other competing products (Figures 1 and 2), and the emission of chemiluminescence can retain up to 12 hours (Figure 3). Due to its strong luminescence signals, the time required exposing to X-ray films or other imaging acquisition systems can be shortened, and this improves the experimental results by decreasing background noises in Western blot (Figure 4).

Highlights

Higher sensitivity : detect target proteins accurate to an even lower pg levels (Figures 1 and 2)

Long duration : retain signals up to 78% of maximal intensity after a 60-min reaction (Figure 3)

Antibody saving : generate strong signals with minimal usage of antibody (Figure 4)

Low crosstalk : reduce background noises and non-specific signals from irrelevant proteins

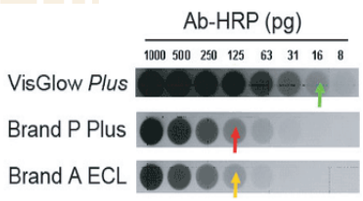


Figure Comparison of sensitivity for VisGlow™ plus, brand P Plus and brand A chemiluminescent substrate. Two fold serially diluted HRP from 1000 to 8pg was used for evaluation. After incubating with the chemiluminescent substrate for 5 minutes, signals were captured by exposure to an X-ray film for 5 seconds. Arrows indicate the samples with comparable intensity. It was found that the chemiluminescent signals from VisGlow™ plus was 8-fold stronger than that of other brands tested.

Characteristics of VisGlow™ *plus* Chemiluminescent Substrate, HRP

Duration of Luminescence	>6 hours
Detection Method	X-ray films or imaging acquisition systems
Typical Antibody Dilution	Primary: 1:1,000–1:20,000 Secondary: 1:40,000–1:200,000
Shelf Life	6 months
Recommended Initial Exposure Time	10 seconds (to films)
Storage Conditions	2–8 °C

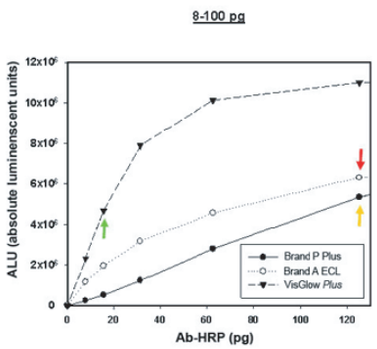


Figure 2: Densitometric analysis of the result in Figure 1 from 0 to 125 pg of HRP.The chemiluminescent signals from VisGlow™ plus was 8-fold stronger than that of other brands tested.

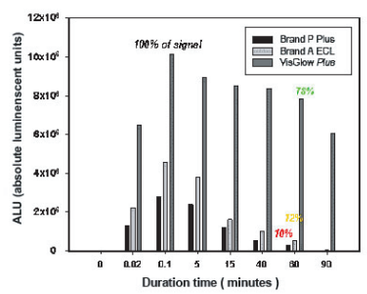


Figure 3: The duration test for VisGlow™ plus, brand P Plus and brand A chemiluminescent substrate. After a 60-min reaction, emission of chemiluminenscence from VisGlow™ plus retained up to 78% of its original intensity while only 10-12% was retained for other brands tested.

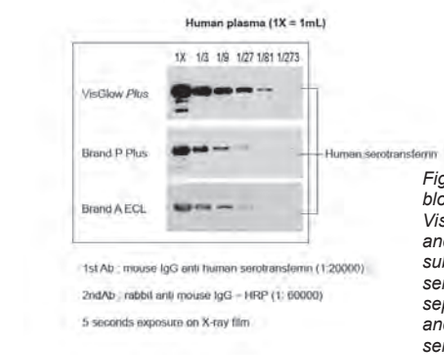


Figure 4: Comparison of Western blot (WB) application for VisGlow™ plus, brand P Plus and brand A chemiluminescent substrate. Human plasma at 1/3 serial dilution from 1 ?l was separated by 12.5% SDS-PAGE and probed using anti-human serotransferrin. All results showed were exposed to X-ray films for 5 seconds.

LuminolPen™, HRP System

LuminolPen™: *a Perfect companion for chemiluminescent detection in Western blot*

Western blot

Western blot is currently the most utilized technique in life science research. After the electrophoresis, gel proteins are transferred onto the nitrocellulose or PVDF membranes then probed with specific primary antibodies and enzyme-conjugated secondary antibodies. To monitor membrane transfer efficiency or estimate molecular weights of the target proteins, the pre-stained protein molecular weight standard marker (pre-stained marker) is always loaded in parallel with protein samples during electrophoresis.

Problems regarding chemiluminescent detection in Western blot

Chemiluminescent detection has been an increasing trend in Western blot experiments. Compared to the conventional colorimetric detection procedures, it has 10 to 100-fold lower detection limit. Generally, after dispensing the enhanced chemiluminescent substrate onto the transferred membrane, the chemiluminescent signals can be either captured by X-ray films or imaging acquisition systems. However, the pre-stained marker will not be developed because the marker proteins will not be hybridized with most primary and secondary antibodies. Many laboratories align the developed images on film with the transferred membrane to indicate the position of marker. However, this procedure is tedious and often causes estimation bias.

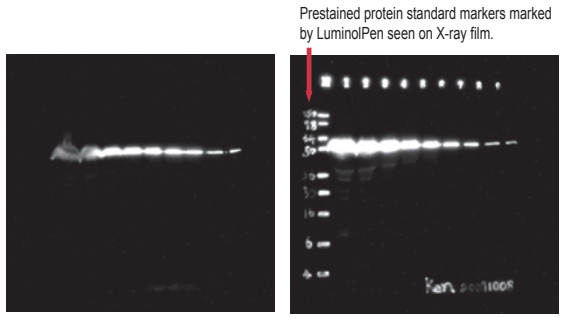
Why you need LuminolPen™ in Western blot?

LuminolPen™ is designed to overcome the limitation mentioned previously. Using LuminolPen™, the position of the pre-stained marker can be easily visualized in a chemiluminescence-based Western blot experiment. Therefore, researchers can observe the position of the prestained marker and the protein signals simultaneously on a single image. There are three advantages for using LuminolPen™.

Observe your pre-stained marker in ECL experiments :
After chemiluminescent development, the position of pre-stained marker marked by LuminolPen™ can be visualized and recorded for further evaluations (Figure 1). Molecular weight of the target proteins can be easily estimated.

Quality control (QC) your chemiluminescent substrate :
LuminolPen™can be taken as an internal control to ensure the chemiluminescent substrate you use is still functional. Signals from the area marked by LuminolPen™ indicate that the chemiluminescent substrate is functional.

Take notes where necessary :
You can use LuminolPen™ to make your experimental notes on the transferred membrane. The blue ink allows researchers to easily mark (1) the size of pre-stained marker, (2) the lane numbers and (3) other important experimental parameters.



Western blot results

Figure1: Left image, the conventional chemiluminescence image; Right image, the chemiluminescence image using the LuminolPen™ to indicate the pre-stained marker.

Just draw it!

After transferring gel proteins onto the nitrocellulose or PVDF membranes and before the procedure of blocking, use the LuminolPen™ to mark the pre-stained marker and perform the following hybridization procedures (Figure 2).

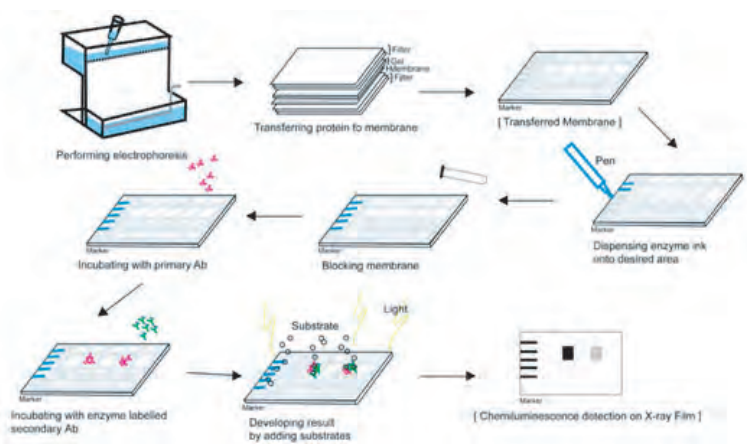


Figure 2: Application of the LuminolPen™ in Western blot.

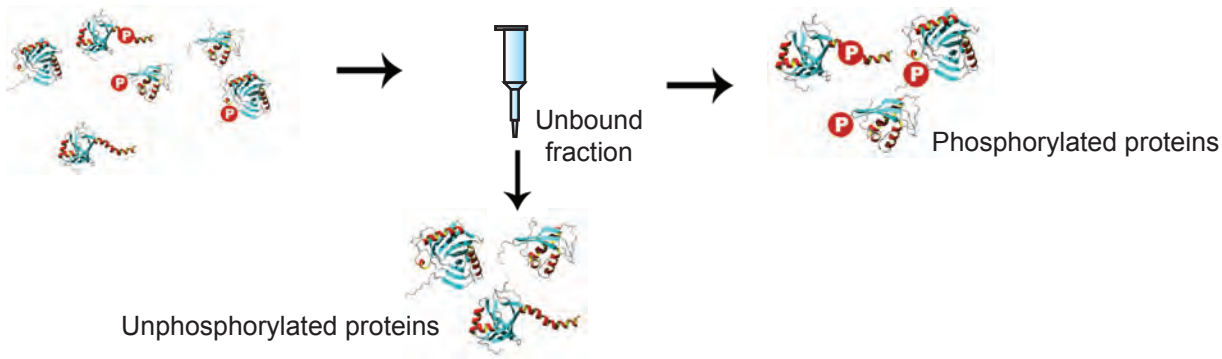
Note: The Pentel™ Tradio Stylo TRJ50 is used as a carrier only. The LuminolPen™ is developed by Visual Protein Biotechnology Corp and there is no association with the Pentel Co., Ltd.

VISUAL PROTEIN

PhosPRO™ Phosphoprotein Purification Kit

Coming up, Phosphoproteomics!

Be the first explorer in the post-proteomic era!!



Characteristics of the PhosPRO™ Phosphoprotein Purification Kit

Immobilized metal ion affinity chromatography (IMAC) is known as an effective method for enriching phosphoproteins. Theoretically, it purifies proteins containing phosphoserine, phosphothreonine and phosphotyrosine residues. However, many IMAC-based phosphoprotein purification procedures suffer from low recovery of phosphoproteins or significant co-purification of non-phosphoproteins.

1. Excellent phosphoprotein purification efficacy

PhosPRO™ Phosphoprotein Purification Kit, our newly developed IMAC-based purification kit, delivers excellent purification results for phosphoproteins. As shown in Figure 1A, when the standard protein mixture is taken to the test, the PhosPRO™ Phosphoprotein Purification Kit specifically enriches the phosphoprotein ovalbumin from other non-phosphoproteins. Almost none of the non-phosphoproteins were co-purified in the elution fraction. With our proprietary chromatographic technology, the PhosPRO™ Phosphoprotein Purification Kit delivers excellent purification efficacy of phosphoprotein in comparison to other commercially available stain kits (Figure 1B) or other reported methods (e.g. Al(OH)3, Figure 1C). The efficiency of PhosPRO™ Phosphoprotein Purification Kit to enrich phosphoproteins from different biological materials, such as animal cell lines, has also been successfully demonstrated (Figure 2).

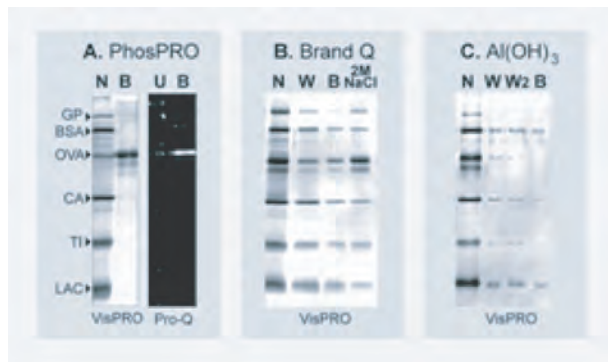


Figure 1. **Purification efficacy from (A) PhosPRO™ Phosphoprotein Purification Kit, (B) Brand Q and (C) colloidal Al(OH)3.** *Protein standard markers containing glycogen phosphorylase b (GP), bovine serum albumin (BSA), ovalbumin (OVA), carbonic anhydrase (CA), trypsin inhibitor (TI) and lactalbumin (LAC) were used for evaluation. Ovalbumin was the only phosphoprotein in the test material. N: flow through; B: eluant; W and W2: washing fraction; 2M NaCl: additional washing step not suggested by manufacturer of Brand Q. The VisPRO™ 5 minute Protein Stain Kit (VisPRO) and Pro-Q Diamond stain (Pro-Q, Invitrogen) were used for developing total proteins or phosphoproteins.*

2. Tunable experimental condition for best purification results

Consider to the complexity and diversity of proteomes from different biological materials, results of purification would vary for individual samples. The PhosPRO™ Phosphoprotein Purification Kit provides a stringent buffer stock for optimizing chromatographic washing conditions. Researchers can prepare their own washing buffer to minimize the binding of non-phosphoproteins and enhance the binding of phosphoproteins.

Choose the purification format depending on your specific requirement

Now, the PhosPRO™ Phosphoprotein Purification Kit has two versions for different applications.

A. PhosPRO™ Phosphoprotein Purification Kit – Pre-packed column: designed for the researchers intending to investigate areas ranging from proteomics to phosphoproteomics. The optimized chromatographic conditions should fulfil most needs. The convenient pre-packed column enables quick purification of phosphoproteins (within 1 hour) and purified phosphoproteins can be directly subjected to the analysis by two-dimensional electrophoresis or mass spectrometry.

B. PhosPRO™ Phosphoprotein Purification Kit – Evaluation : designed for the advanced research in phosphoproteomics. Researchers can optimize their own chromatographic conditions, such as binding capacity or washing stringency by using the provided buffer.

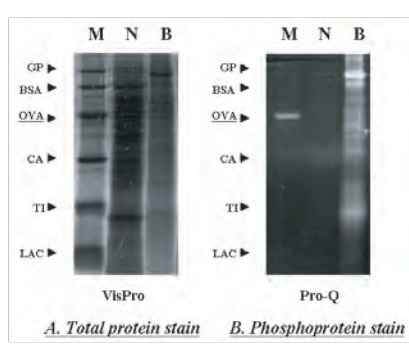


Figure 2. **Purification of phosphoproteins from mouse myeloma SP2/0 cell lysates.** *1mg protein extracted from the mouse myeloma SP2/0 cell lysates were dissolved in 2mL system buffer and purified by a Per-packed column (PP401-C-6. The purification results was evaluated by staining the 12.5% SDS-PAGE gels using A. VisPRO™ 5 minutes Protein Stain Kit (VisPRO) and B. Pro-Q Diamond stain (Pro-Q, Invitrogen) for total proteins and phosphoproteins respectively. N: the unbound flow-through. B: The eluted fractions. M: Low molecular weight marker (GE Healthcare). The 45 kDa ovalbumin (Ova) is known as the only phosphoproteins in the low molecular weight marker.*

VISUAL PROTEIN

ImmunoFast™ Adjuvant

No toxicity and harmful adverse effects.
Most friendly to your immunized animals
Just mix the **ImmunoFast™** *with the antigen for 5 minutes*
No loss of residual emulsifying solution in syringes

Visual Protein introduces a new aqueous emulsifying adjuvant, **ImmunoFast™** *which is specifically designed to accelerate and enhance immunoresponse in research animals. The* **ImmunoFast™** *Adjuvant is an attractive alternative of the Complete Freund's Adjuvant (CFA) and other adjuvants, in terms of safety and efficacy.*

Enchanting " 3S " Characteristics

Speedy induction of antibodies

The most attractive character of ImmunoFast™ is its efficiency to elicit a significant immunoresponse in a very short period of time, even for the antigens difficult to elicit an immunoresponse. Within 2 weeks after the first immunization, the ImmunoFast™(Figure 1, closed circle) elicits a 100,000-fold stronger immunoresponse in animals than CFA (Figure 1, opened circle). Employing ImmunoFast™ can induce a significant immunoresponse within 2 weeks (Figure 2, closed circle), whereas the conventional CFA may take at least 4 weeks to elicit an equivalent immunoresponse (Figure 2, opened circle). Furthermore, as for ImmunoFast™, a plateau of immunoresponse will be reached at the 4th week (Figure 2, green arrow). The ImmunoFast™ efficiently makes the best of your time for research without compromising antibody titer.

Strong immunoresponse

The ImmunoFast™ can elicit a much stronger immunoresponse in animals than other adjuvants such as the CFA. After performing the regular immunization procedure in animals (4 boosts within 8 weeks), the immunoresponse induced by ImmunoFast™ (Figure 2, at 8th week, blue arrow) is approximately 10-fold stronger than that induced by CFA.

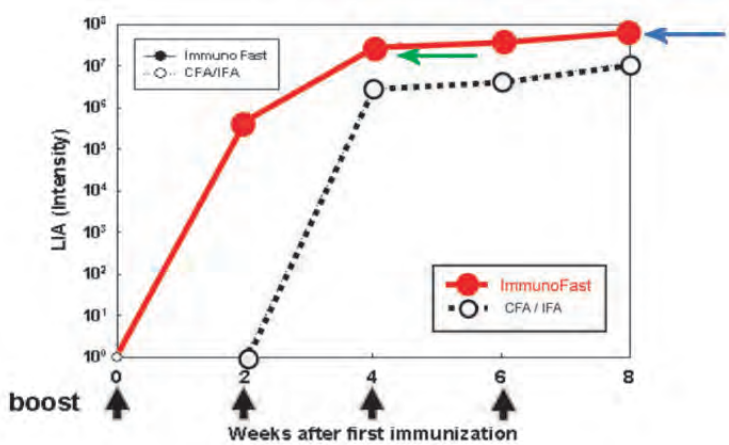


Figure 2: Time required for ImmunoFast™ *and CFA/IFA to induce an appreciable immunoresponse.*

Switch IgM to IgG efficiently

The ImmunoFast™ can induce a complete class switch from IgM to IgG in immunized animals (Figure 3). The ImmunoFast™ elicits more profitable immunoglobulin molecules (IgG) for downstream applications.

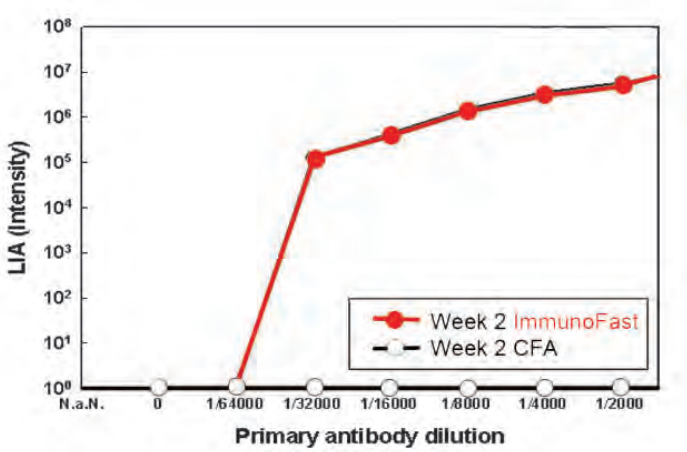


Figure 1: Comparison of the immunoresponse elicited by ImmunoFast™ and CFA at the 2nd week after the first immunization.

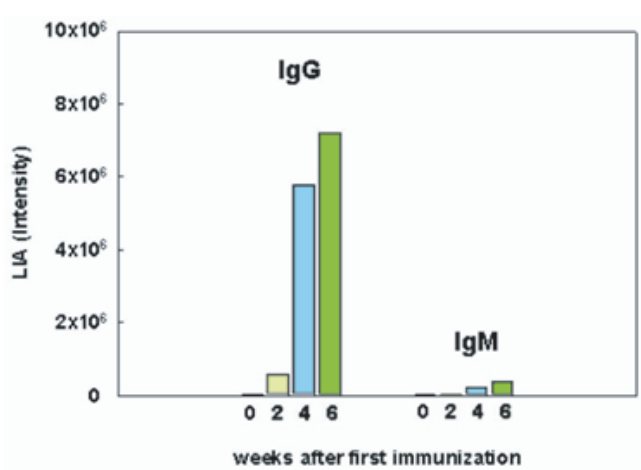


Figure 3: Levels of the elicited immunoglobulin molecules in an ImmunoFast™ boosted animal.