VisPRO 5 minutes Protein Stain Kit

VISUAL PROTEIN

Did you have the following problem in your experiment ?

- SDS-PAGE staining
  - **Q1 A long time to staining: SDS-PAGE stained spend 2 hours to over night. All of stain can't immediately get staining result.**
  - **Q2 Low sensitivity: the Commassie blue stain, the sensitivity might be to 50ng. We hope to get more sensitivity staining?**

VISUAL PROTEIN

Did you have the following problem in your experiment ?

- SDS-PAGE staining
  - **Q3 the complex protocol in staining preparation： Sliver stain, it's high sensitivity staining. But it has complex step, stained for a long time, and not easy to operating. It is easy to be fail and re-do it. We always hope to has high sensitivity and easy staining method.**

  - **Q4 MASS not compatible : To identify protein , we always use MALDI-TOF or LS/MS/MS, but commonly used stain can interfere with MASS result. Even mass-compatible silver staining also reduce the success rate of identification.**

VISUAL PROTEIN

Did you have the following problem in your experiment ?

- SDS-PAGE staining
  - **Q5 Can’t estimate the pattern of electrophoresis before transferring: Before transferring, we can’t check the pattern of electrophoresis. If the pattern is bad, we can’t check it. And It is always waste time. We need a new staining method can make SDS-PAGE transferring.**

- **Q6 Recovery activity protein in gel: When recovery protein, we need to use CBR stain or other stain to check protein’s position. But all commonly used stain cause protein loss activity. So we need a new stain can recovery protein and keep protein activity.**

VISUAL PROTEIN

Did you have the following problem in your experiment ?

- SDS-PAGE staining
  - **Q7 Expensive. To Consider the cost of reagents and the cost of time, VisPRO staining can obtain the higher C/P value.**
  - **Q8Using SYPRO Ruby stain, spotting protein must use UV to excite gel, it is a great burden on the eye. When using Cydye, It is not clear to saw the spot and inconvenient to select protein spot. Is there any suggestion in safety and convenience?**

VISUAL PROTEIN

The fastest stain

Q1 A long time to staining: SDS-PAGE stained spend 2 hours to over night. All of stain can't immediately get staining result.

VisPRO Stain is the fastest stain . it just spends 15 min obtaining the result.

Protocol for VisPRO 5 minutes Protein Stain Kit

1. add Solution 1 and shack 5 mins
2. Discard Solution 1, quickly wash it
3. add Solution 2 and shack 30 sec
4. discard Solution 2 and quickly wash twice

VISUAL PROTEIN

Comparison staining time

VisPRO Stain costs shorten time

Methods	VisPRO Stain	Sypro™ Ruby	Silver Stain	CBR Stain
Preparation of solutions	0 min	5 min	20 min	0 min
Fixation	0 min	1 hr	1hr~overnight	0 min
Staining procedure	5~15 min	30 min~ o/n	2.5~4 hr	30 min~ o/n
Destaining procedure	0 min	1 hour	0 min	2 hr~ o/n
Total	5 ~15 min	2.5~o/n	3.5~0/n	2.5 hr~ o/n

⑩ **VisPRO stain**

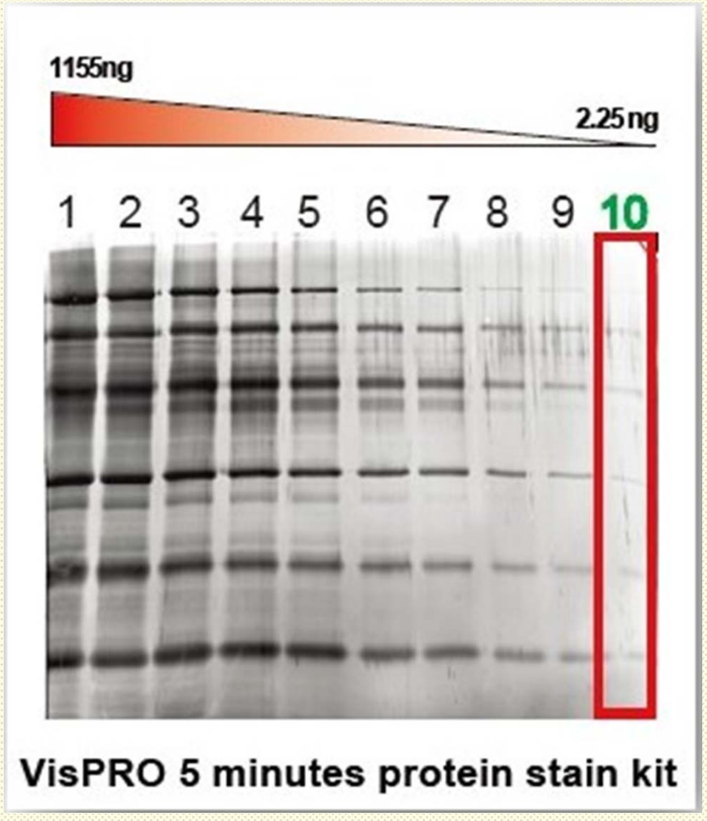
Only spends 5~15 min.

The fastest stain in the market

VISUAL PROTEIN

Excellent Sensitivity

– **Q2 Low sensitivity: the Commassie blue stain, the sensitivity might be to 50ng. We hope to get more sensitivity staining?**



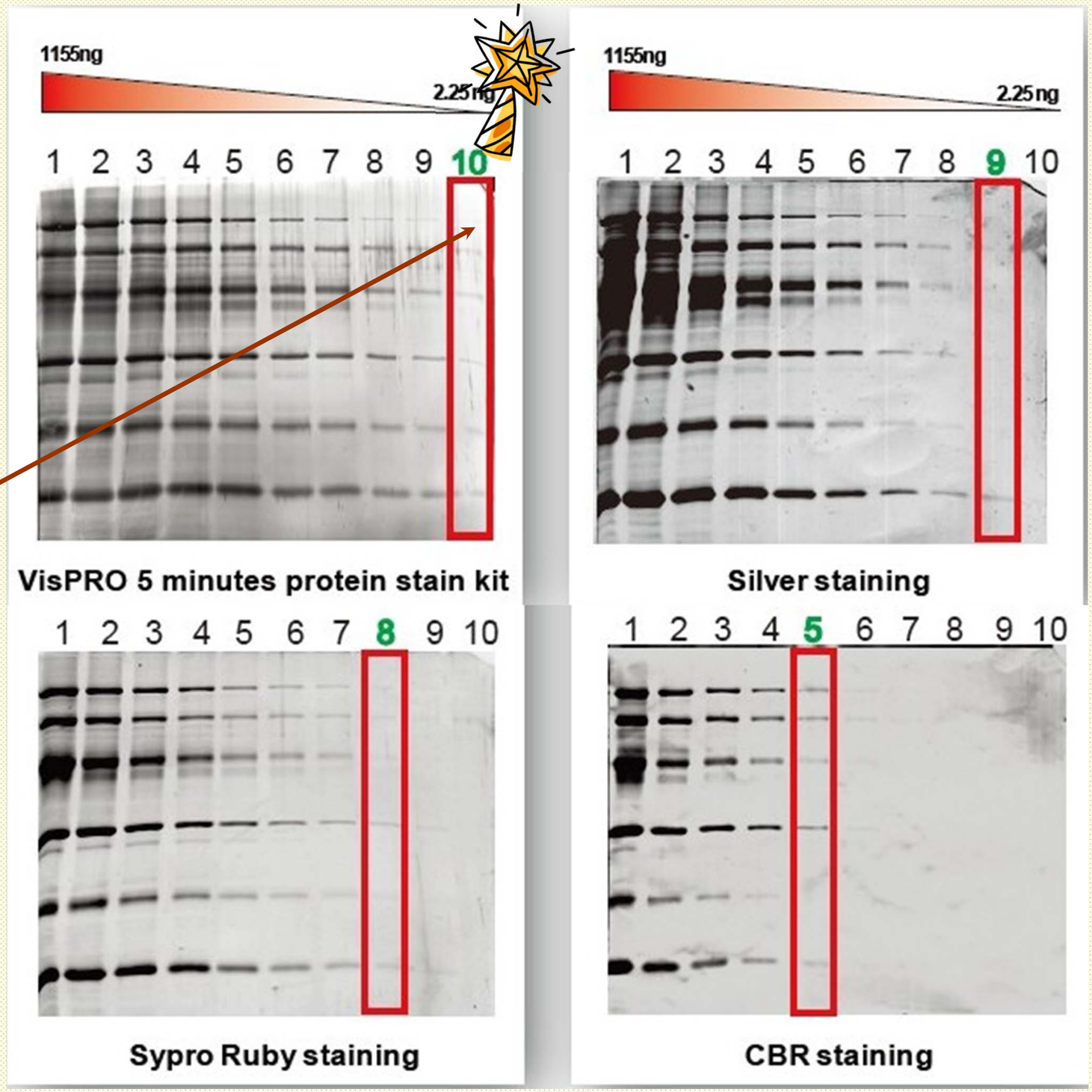
VisPRO 5 minutes protein stain kit

VisPRO detects protein levels accurate to 1 ng as sensitivity as SYPRO Ruby and Sliver stain.

VISUAL PROTEIN

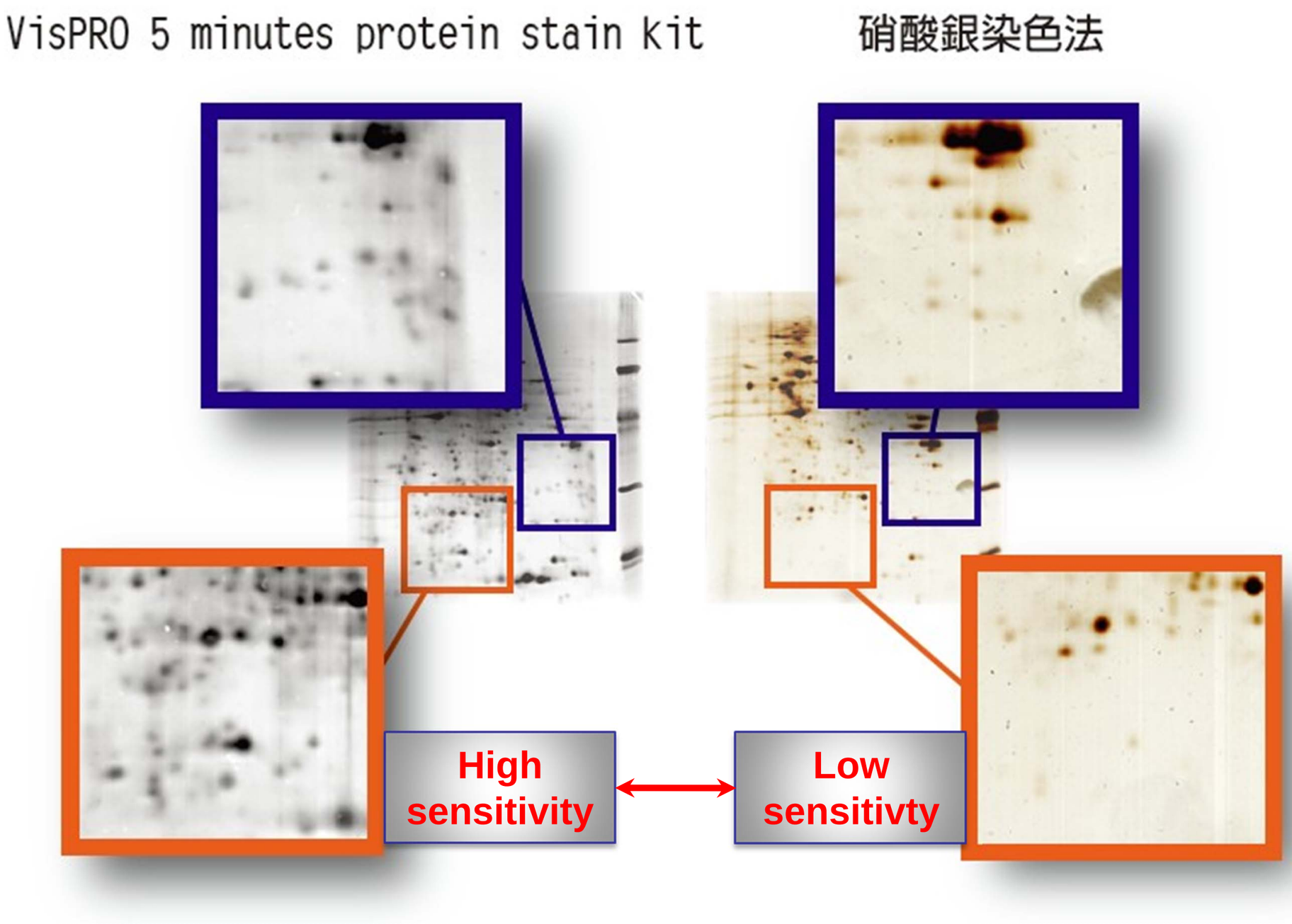
Comparison of staining sensitivity-VisPRO Stain is the best

Comparison of staining sensitivity : even as little as 1ng of protein can be detected



VISUAL PROTEIN

Comparison of staining results :VisPRO Stain VS sliver stain



VISUAL PROTEIN

Easy to use VisPRO Stain

– **Q3 the complex protocol in staining preparation： Sliver stain, it's high sensitivity staining. But it has complex step, stained for a long time, and not easy to operating. It is easy to be fail and re-do it. We always hope to has high sensitivity and easy staining method.**

VisPRO Stain, Two reagents, Four steps, 5-15 mins

Protocol for VisPRO 5 minutes Protein Stain Kit

1. add Solution 1 and shack 5 mins
2. Discard Solution 1, quickly wash it
3. add Solution 2 and shack 30 sec
4. discard Solution 2 and quickly wash twice

VISUAL PROTEIN

The Best MASS Choice

Q4 MASS not compatible : To identify protein , we always use MALDI-TOF or LS/MS/MS, but commonly used stain can interfere with MASS result. Even mass-compatible silver staining also reduce the success rate of identification.

VisPRO Stain is a reverse staining method

1. **Reagents don't combine with proteins, and the molecular weight would be change.**

2. **Discard reagent easily, non-interference in the MASS result**

3. **Recommended by** authoritative papers, rising the success rate in identification
   （**ie. Journal of Proteome Research Vol. 8, No. 10, 2009 4393**）

4. **Recommended by many professors.**

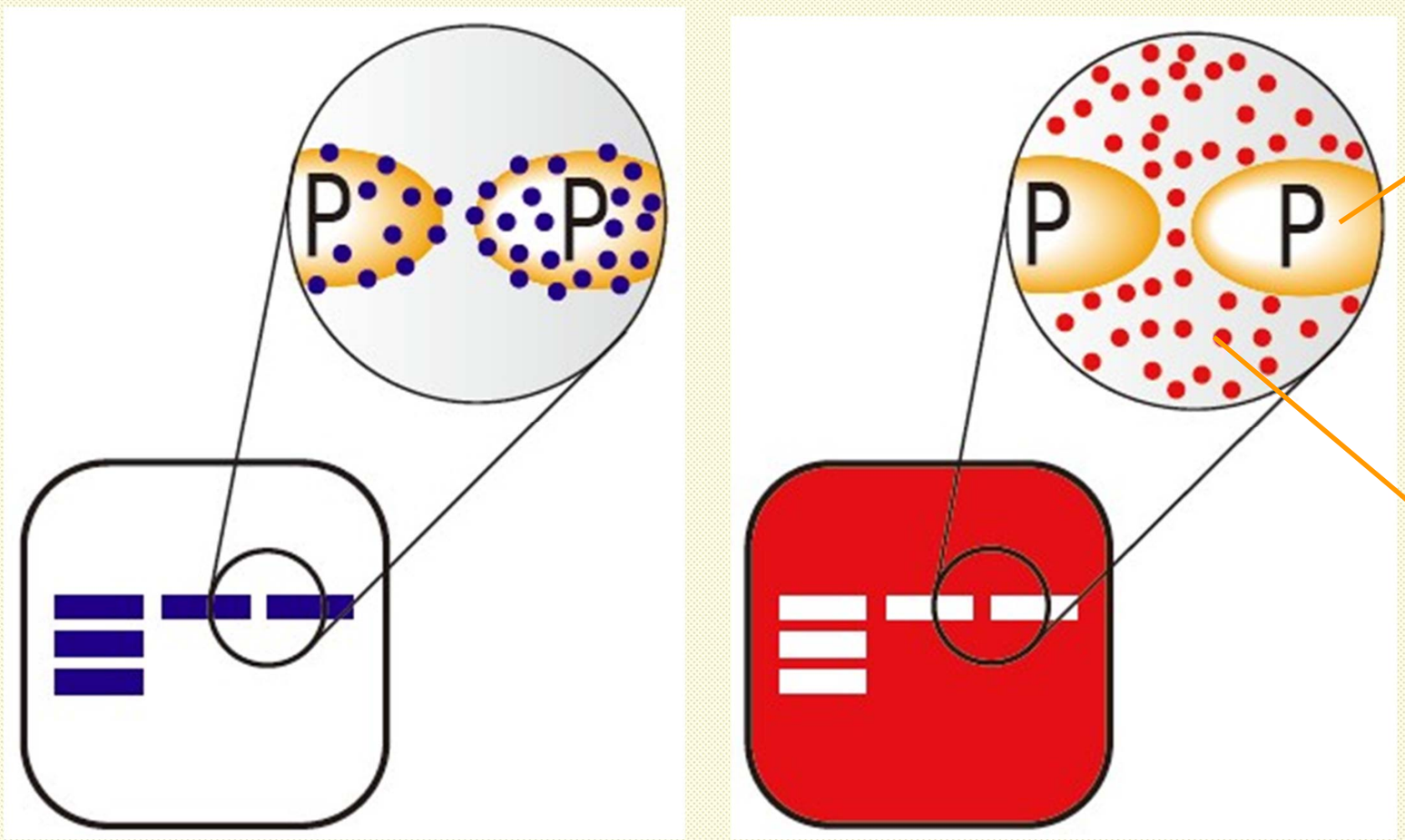
VISUAL PROTEIN

What’s Reverse Staining ?

(VisPRO 5 minutes Protein Stain Kit)

PostiveStain

Reverse Stain



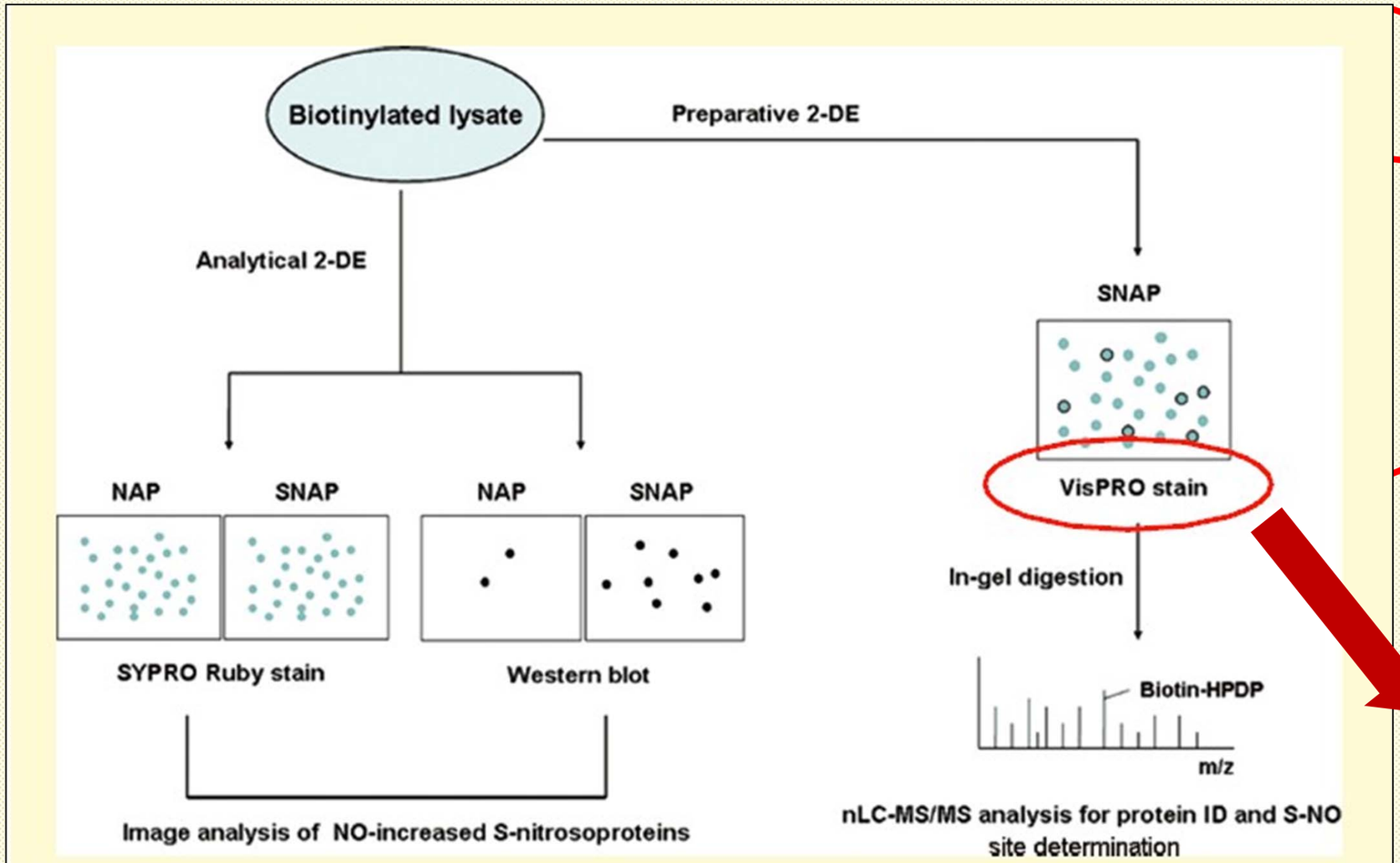
Macromolecules form Space obstacles

Small insoluble white powder left Zinc-imidazole complex

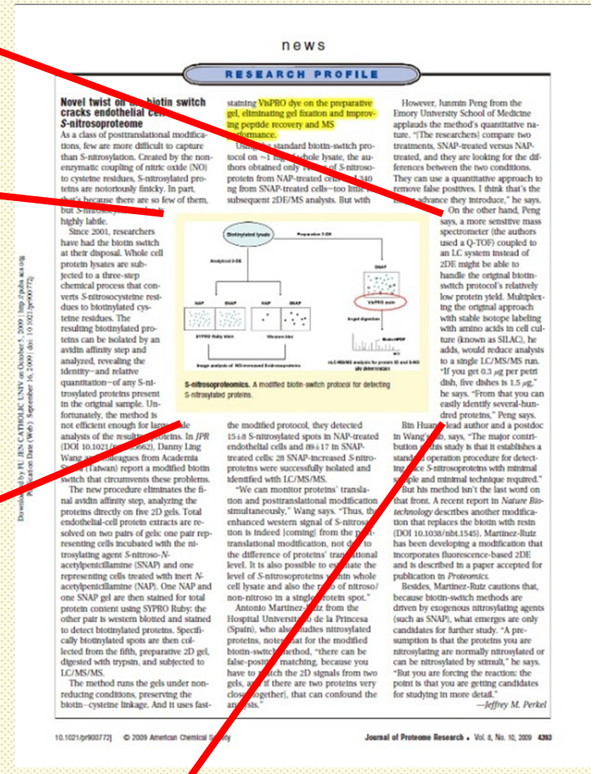
Reverse Stain: Stain non-protein part in gel and enhance the target protein by background

VISUAL PROTEIN

Journal of Proteome Research, Aug. 2009.
Research Profile



S-nitrosoproteomics. A modified biotin-switch protocol for detecting S-nitrosylated proteins.



news

RESEARCH PROFILE

Novel twist on the biotin switch cracks endothelial *S*-nitrosoproteome

As a class of posttranslational modifications, few are more difficult to capture than S-nitrosylation. Created by the nonenzymatic coupling of nitric oxide (NO) to cysteine residues, S-nitrosylated proteins are notoriously finicky. In part, that's because there are so few of them, but S-nitrosylation is also highly labile.

Since 2001, researchers have had the biotin switch at their disposal. Whole cell protein lysates are subjected to a three-step chemical process that converts S-nitrosocysteine residues to biotinylated cysteine residues. The resulting biotinylated proteins can be isolated by an avidin affinity step and analyzed, revealing the identity—and relative quantitation—of any S-nitrosylated proteins present in the original sample. Unfortunately, the method is not efficient enough for large-scale analysis of the resulting proteins. In *JPR* (DOI 10.1021/[illegible]2662), Danny Ling Wang and colleagues from Academia Sinica (Taiwan) report a modified biotin switch that circumvents these problems.

The new procedure eliminates the final avidin affinity step, analyzing the proteins directly on five 2D gels. Total endothelial-cell protein extracts are resolved on two pairs of gels: one pair representing cells incubated with the nitrosylating agent *S*-nitroso-*N*-acetylpenicillamine (SNAP) and one representing cells treated with inert *N*-acetylpenicillamine (NAP). One NAP and one SNAP gel are then stained for total protein content using SYPRO Ruby; the other pair is western blotted and stained to detect biotinylated proteins. Specifically biotinylated spots are then collected from the fifth, preparative 2D gel, digested with trypsin, and subjected to LC/MS/MS.

The method runs the gels under nonreducing conditions, preserving the biotin–cysteine linkage. And it uses fast-staining VisPRO dye on the preparative gel, eliminating gel fixation and improving peptide recovery and MS performance.

Using the standard biotin-switch protocol on ~1 mg whole lysate, the authors obtained only traces of S-nitrosoprotein from NAP-treated cells—too little for subsequent 2DE/MS analysis. But with the modified protocol, they detected 15±8 S-nitrosylated spots in NAP-treated endothelial cells and 89±17 in SNAP-treated cells; 28 SNAP-increased S-nitroproteins were successfully isolated and identified with LC/MS/MS.

"We can monitor proteins' translation and posttranslational modification simultaneously," Wang says. "Thus, the enhanced western signal of S-nitrosylation is indeed [coming] from the post-translational modification, not due to the difference of proteins' translational level. It is also possible to evaluate the level of *S*-nitrosoproteins within whole cell lysate and also the ratio of nitroso/non-nitroso in a single protein spot."

Antonio Martínez-Ruiz from the Hospital Universitario de la Princesa (Spain), who also studies nitrosylated proteins, notes that for the modified biotin-switch method, "there can be false-positive matching, because you have to match the 2D signals from two gels, and if there are two proteins very close together, that can confound the analysis."

However, Junmin Peng from the Emory University School of Medicine applauds the method's quantitative nature. "[The researchers] compare two treatments, SNAP-treated versus NAP-treated, and they are looking for the differences between the two conditions. They can use a quantitative approach to remove false positives. I think that's the big advance they introduce," he says.

On the other hand, Peng says, a more sensitive mass spectrometer (the authors used a Q-TOF) coupled to an LC system instead of 2DE might be able to handle the original biotin-switch protocol's relatively low protein yield. Multiplexing the original approach with stable isotope labeling with amino acids in cell culture (known as SILAC), he adds, would reduce analysis to a single LC/MS/MS run. "If you get 0.3 μg per petri dish, five dishes is 1.5 μg," he says. "From that you can easily identify several-hundred proteins," Peng says.

Bin Huang, lead author and a postdoc in Wang's lab, says, "The major contribution of this study is that it establishes a standard operation procedure for detecting the S-nitroproteins with minimal sample and minimal technique required."

But his method isn't the last word on that front. A recent report in *Nature Biotechnology* describes another modification that replaces the biotin with resin (DOI 10.1038/nbt.1545). Martínez-Ruiz has been developing a modification that incorporates fluorescence-based 2DE and is described in a paper accepted for publication in *Proteomics*.

Besides, Martínez-Ruiz cautions that, because biotin-switch methods are driven by exogenous nitrosylating agents (such as SNAP), what emerges are only candidates for further study. "A presumption is that the proteins you are nitrosylating are normally nitrosylated or can be nitrosylated by stimuli," he says. "But you are forcing the reaction: the point is that you are getting candidates for studying in more detail."

—*Jeffrey M. Perkel*

Refer VisPRO Stain—obtaining the best result of MASS

VISUAL PROTEIN

Papers(I)

VisPRO 5 minutes Protein Stain Kit in Papers

- Bin Huang, Shih Chung Chen, and Danny Ling Wang. **Cardiovascular Research** 2009; 83 : 536-546. Shear flow increases S-nitrosylation of proteinsin endothelial cells.

- Ching-Yu Lin, Vinchi Wang, Hao-Ai Shui, Rong-Huay Juang, Ai-Ling Hour, Pei-Sing Chen, Hui-Ming Huang, Szu-Yu Wu, Jen-Chieh Lee, Tzung-Lin Tsai and Han-Min Chen. **Proteomics** 2009; 9:696-709. A comprehensive evaluation of imidazole-zinc reverse stain for current proteomic researches.

- Bin Huang, Chung Ling Liao, Ya Ping Lin, Shih Chung Chen and Danny Ling Wang. **Journal of Proteome Research** 2009; 8: 4835–4843. S-nitrosoproteome in endothelial cells revealed by a modified biotin switch approach coupled with western blot-based two-dimensional gel electrophoresis

VISUAL PROTEIN

Papers (II)

VisPRO 5 minutes Protein Stain Kit in theses

- Yu-Jen Chen, Chih-Chia Yu, Shui-Tein Chen, Tai-Yuan Chen and Hui-Fen Liao. **Proteomics Clin. Appl**. 2009; 3: 563–573. Functional regulation and proteomic characterization of human natural killer cells through recombinant human granulocyte-colony stimulating factor treatment.

- Ching-Yu Lin, Hui-Ming Huang, and Han-Min Chen, **Biotechniques** 2006; 41:560-564. Use of backlit light plate to enhance visualization of imidazole-zinc reverse stained gels.

- Hui-Chung Wu, Chien-Chang Yen, Wen-Huei Tsui, Han-Min Chen. **Analytical biochemistry**, 2009; 03: 2693-2697. A red line not to cross: Evaluating the limitation and properness of gel image tuning procedures.

VISUAL PROTEIN

Papers(I)

- **VisPRO 5 minutes Protein Stain Kit in theses**
- Yi-Jen Liau, Lisa Wen, Jei-Fu Shawc, Chi-Tsai Lin. **Journal of Biotechnology**, 2007 ; 131:84–91. A highly stable cambialistic-superoxide dismutase from Antrodia camphorata: *Exprcamphorata: xpression* in yeast and enzyme properties.

- Shih Chung Chen, Bin Huang, Yu Chi Liu, Kou Gi Shyu, Pen Y. Lin and Danny Ling Wang. **Biochemical and Biophysical Research Communications** 2008 ; 377:1274-1278. Acute hypoxia enhances proteins’ S-nitrosylation in endothelial cells.

- Yasuhiko Kizuka, Kyoko Kobayashi, Shinako Kakuda, Yukari Nakajima, Satsuki Itoh, Nana Kawasaki, and Shogo Oka. **Glycobiology** 2008; 18(4): 331–338. Laminin-1 is a novel carrier glycoprotein for the nons

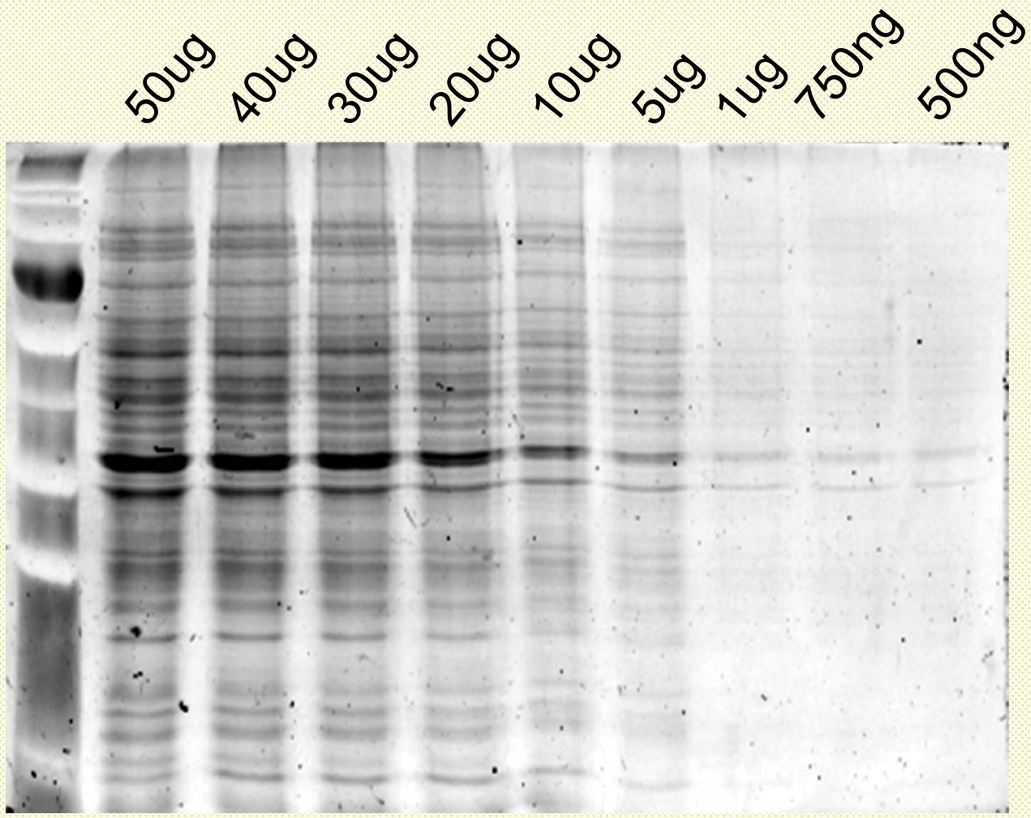
VISUAL PROTEIN

Reference: VisPRO 5 minutes Protein Stain Kit

{**The I**nstrument Center, Institute of Biomedical Sciences, Academia SINICA**}**

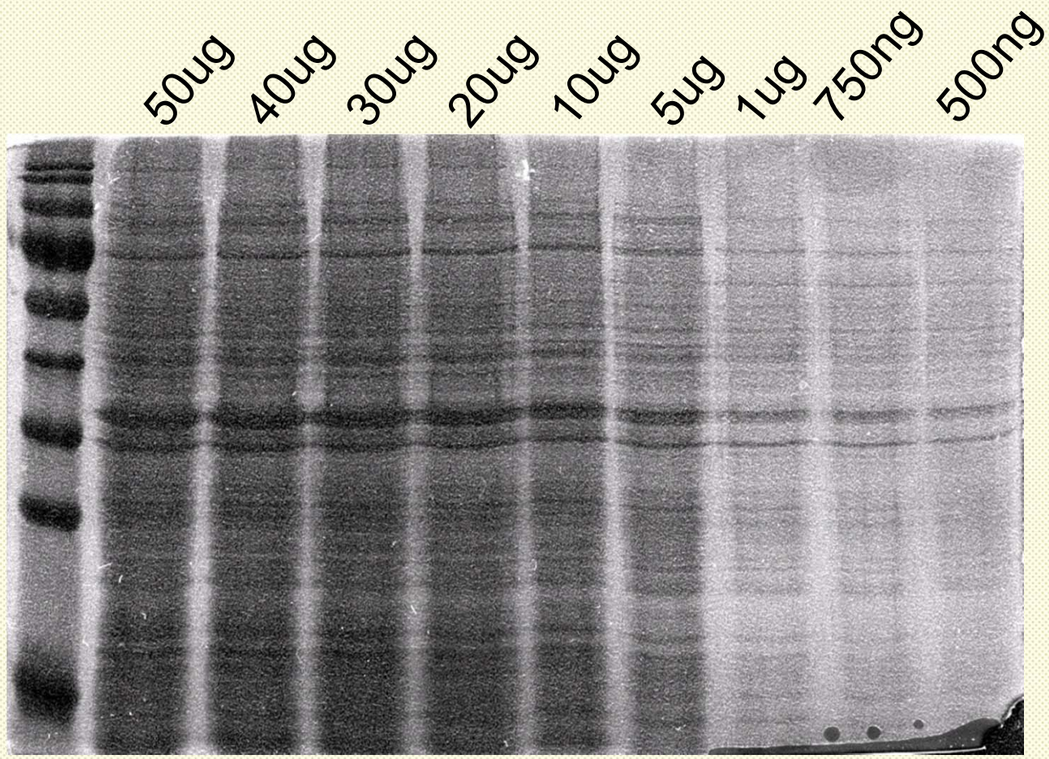
July, 2007

E.Coli-K12 lysate



12% SDS-PAGE
Stain: Sypro Ruby 3 hours

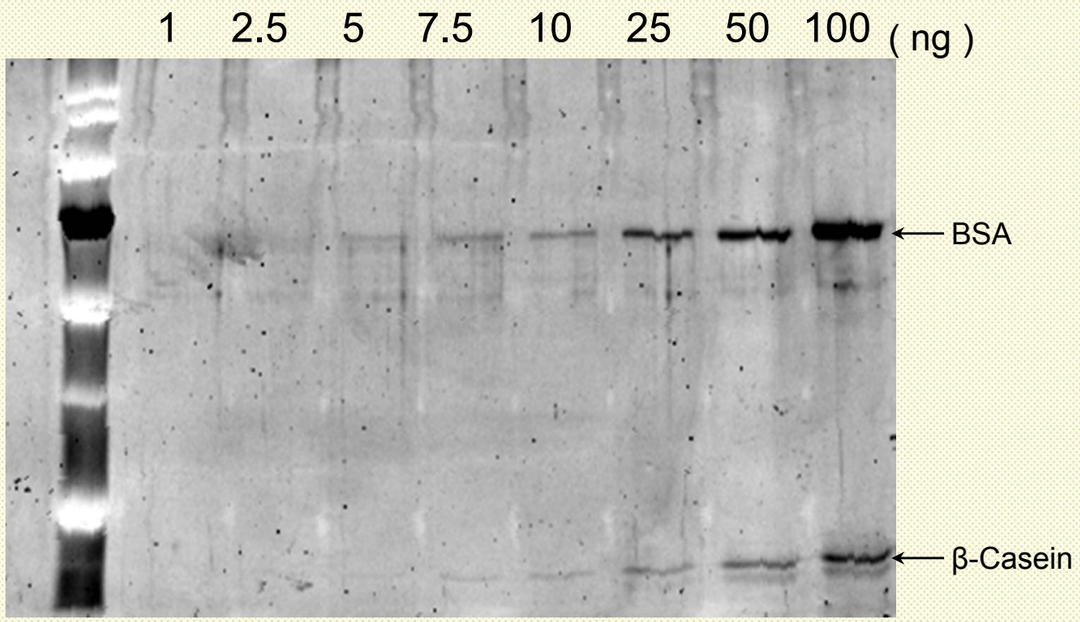
E.Coli-K12 lysate



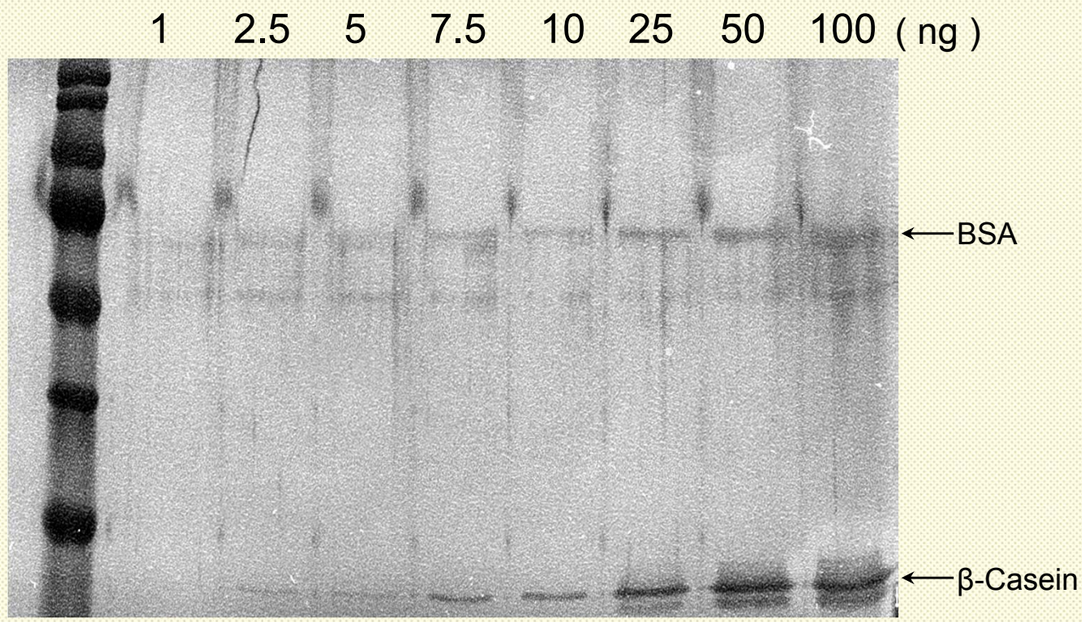
12% SDS-PAGE
Stain: VisPro 5 (< 10 mins)

VISUAL PROTEIN

2007.07.09 Sypro Ruby / VisPro 5 gel staining



Sypro Ruby



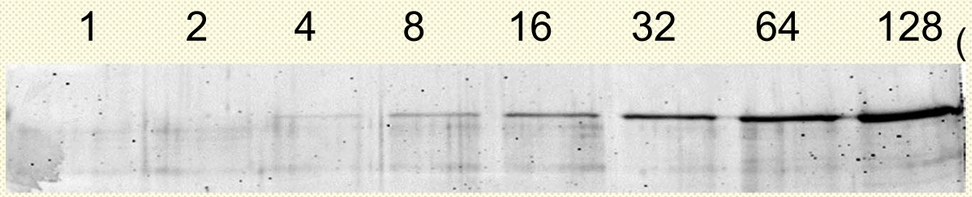
VisPro 5

Run 12% Acrylamide / bis gel
Fixation: 50% Methanol, 7% Acetic acid. 30mins/time, 2 times
Stain: 3 hours
Washing: 10% Methanol, 7% Acetic acid. 30mins, once
Rinse: ddH_2O 5mns/time, 2 times
Keep in ddH_2O 2 nights, then scan the gel by Typhoon9410
Excitation: ~532nm
Emission: 610nm

使用染完Sypro Ruby之gel作染色
1. Balance: Solution I 5 mins
2. Rinse: Rinse the gel with ddH_2O rapidly (1 time)
3. Stain: Solution II, shake the gel gently (less than 30 sec)
4. Rinse: Rinse the gel with ddH2O rapidly (1 time)
5. Keep in ddH_2O , then use scanner to store gel image.

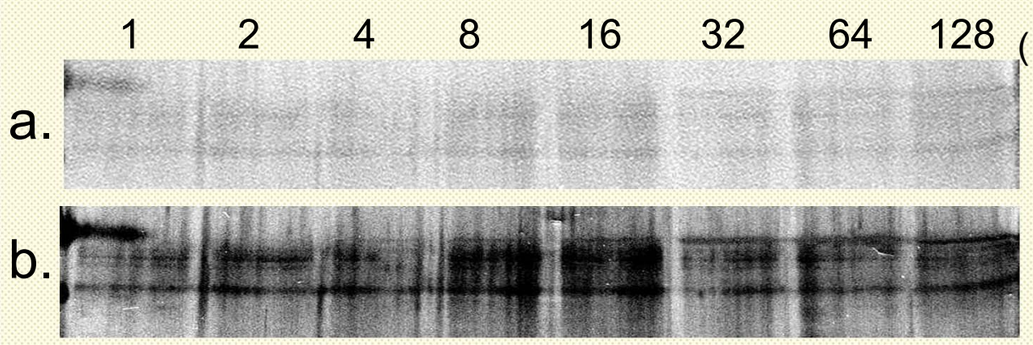
VISUAL PROTEIN

2007.07.013 Sypro Ruby / VisPro 5 gel staining



Sypro Ruby

1.Run 10% Acrylamide / bis gel
2.Sample: BSA (MW.~68KDa)
3.Fixation: 50% Methanol, 7% Acetic acid. 30mins/time, 2 times
4.Stain: 3 hours
5.Washing: 10% Methanol, 7% Acetic acid. 30mins, once
6.Rinse: ddH_2O 5mns/time, 2 times
7.Keep in ddH_2O over night, then scan the gel by Typhoon9410
Excitation: 488nm
Emission: 610nm
＊Use different wavelength to scan gel (457nm, 488nm, 532nm) Obtaining the similar result, but the signal(532nm) is stronger after scaning

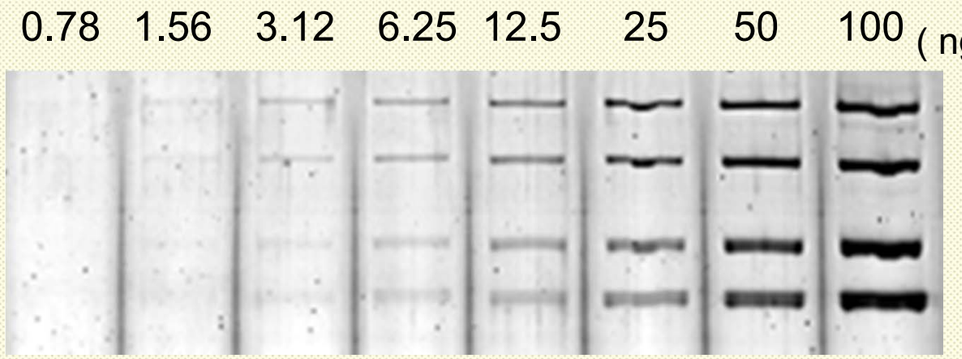


VisPro 5 min protein stain kit

The gel with Sypro Ruby
1. Balance: Solution I 5 mins
2. Rinse: Rinse the gel with ddH_2O rapidly (1 time)
3. Stain: Solution II, shake the gel gently (less than 30 sec)
4. Rinse: Rinse the gel with ddH2O rapidly (1 time)
5. Keep in ddH_2O , then use scanner to store gel image.
Figure a. reflect mode scan
Figure b. Penetration mode scan
＊observing with naked eyes is better than scanner

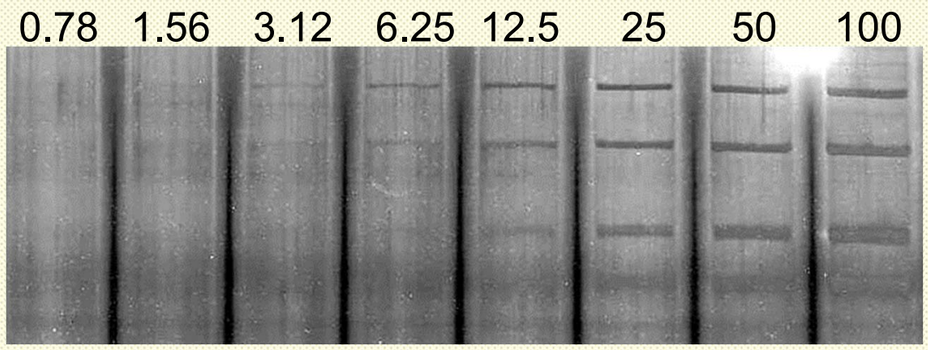
VISUAL PROTEIN

2007.07.30 Sypro Ruby / VisPro 5 gel staining (test BSA degradation)



Sypro Ruby

1.Run 10% Acryl amide / bis gel
2.Sample: BSA (MW.~68KDa)
3.Fixation: 50% Methanol, 7% Acetic acid. 30mins/time, 2 times
4.Stain: over night (~20hr) in RT
5.Washing: 10% Methanol, 7% Acetic acid. 40mins, once
6.Rinse: ddH_2O 5mns/time, 2 times
7.Keep in ddH_2O, then scan the gel with Typhoon9410
Excitation: 532nm
Emission: 610nm

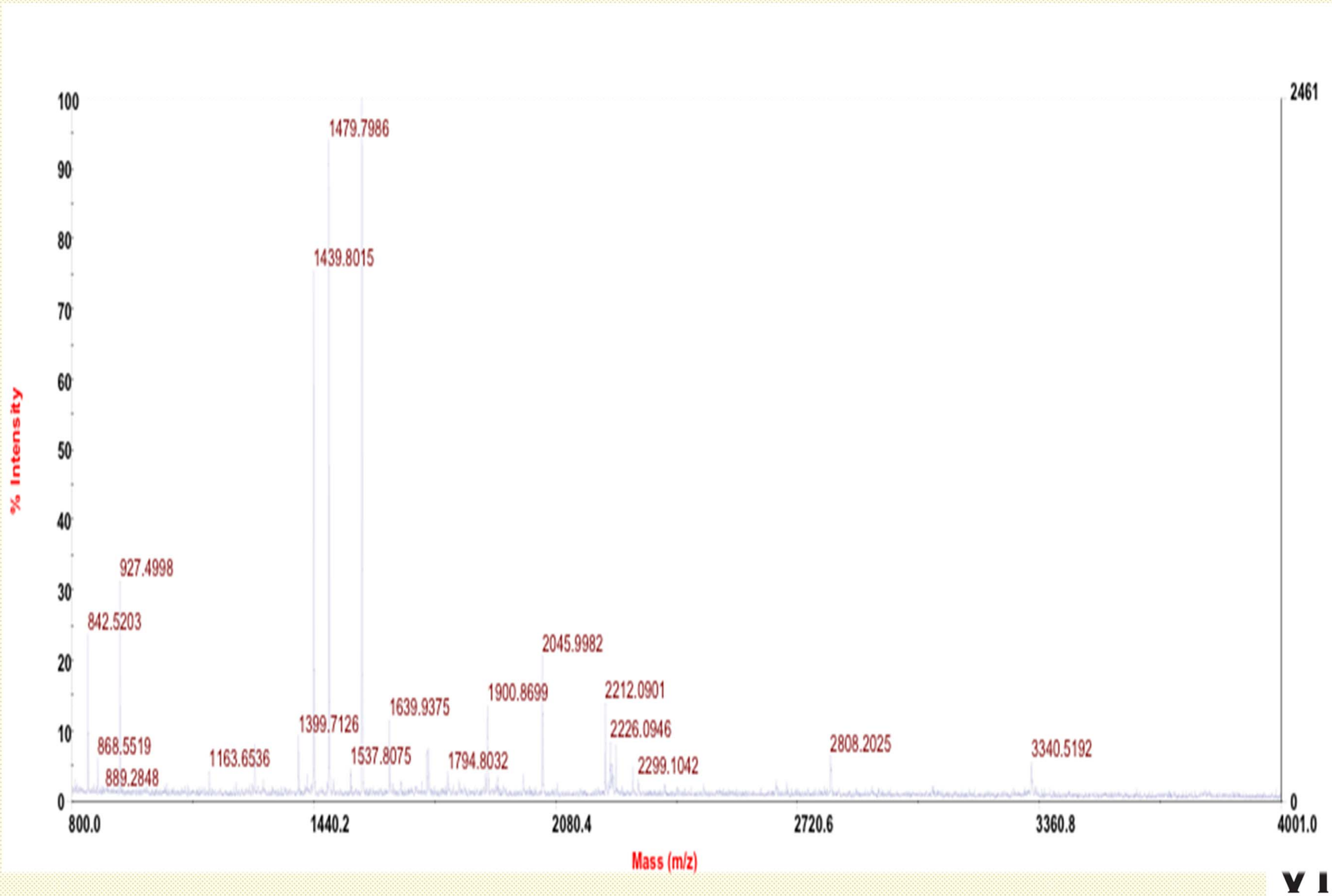


VisPro 5 min protein stain kit

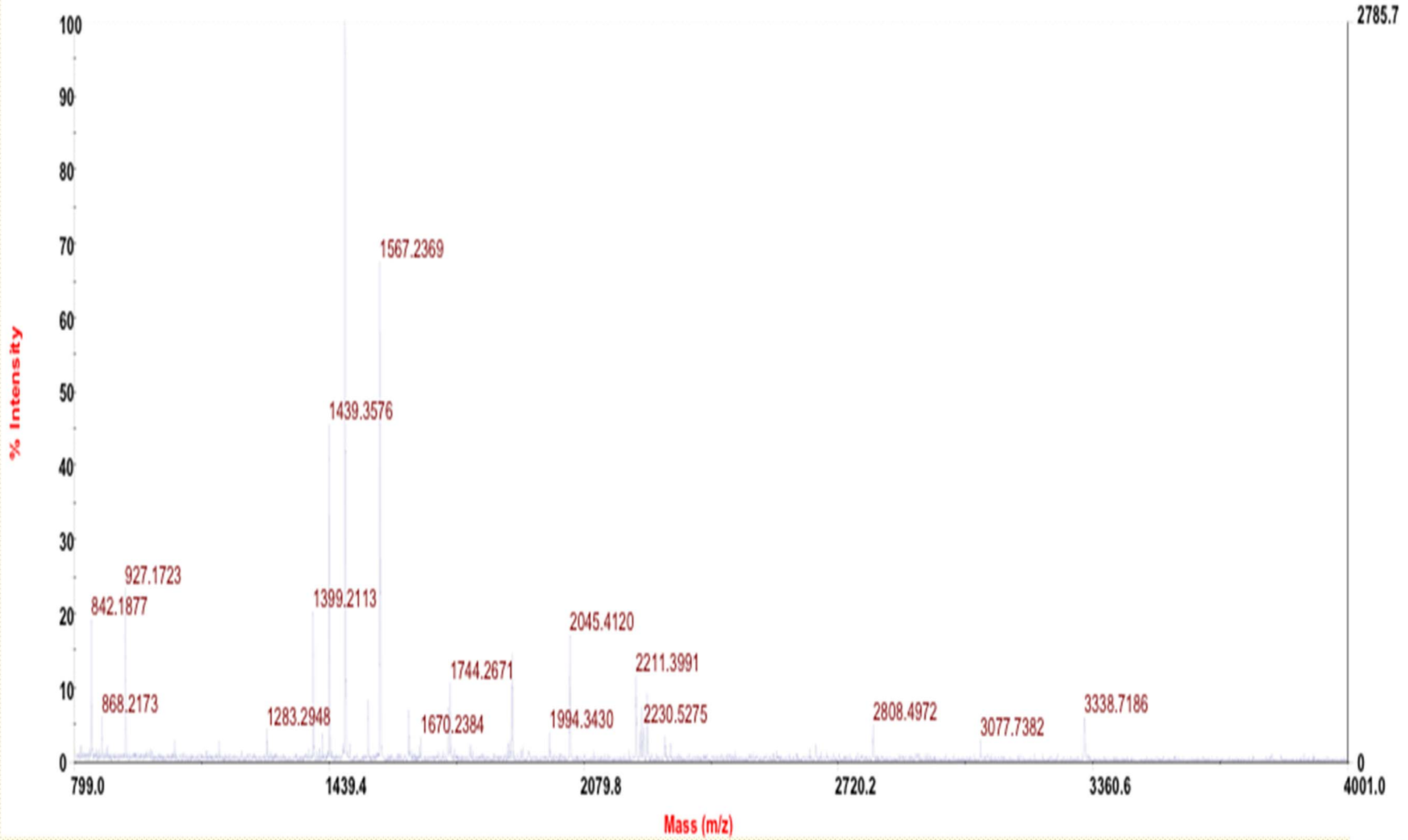
The gel with Sypro Ruby(not the same gel as above)
1. Balance: Solution I 5 mins
2. Rinse: Rinse the gel with ddH_2O rapidly (1 time)
3. Stain: Solution II, shake the gel gently (less than 30 sec)
4. Rinse: Rinse the gel with ddH2O rapidly (1 time)
5. Keep in ddH_2O , then use scanner to store gel image.

VISUAL PROTEIN

In-gel digestion à PMF detected by MALDI-TOF
stain: VisPro 5
100ng BSA
Mass fit research result: 41%

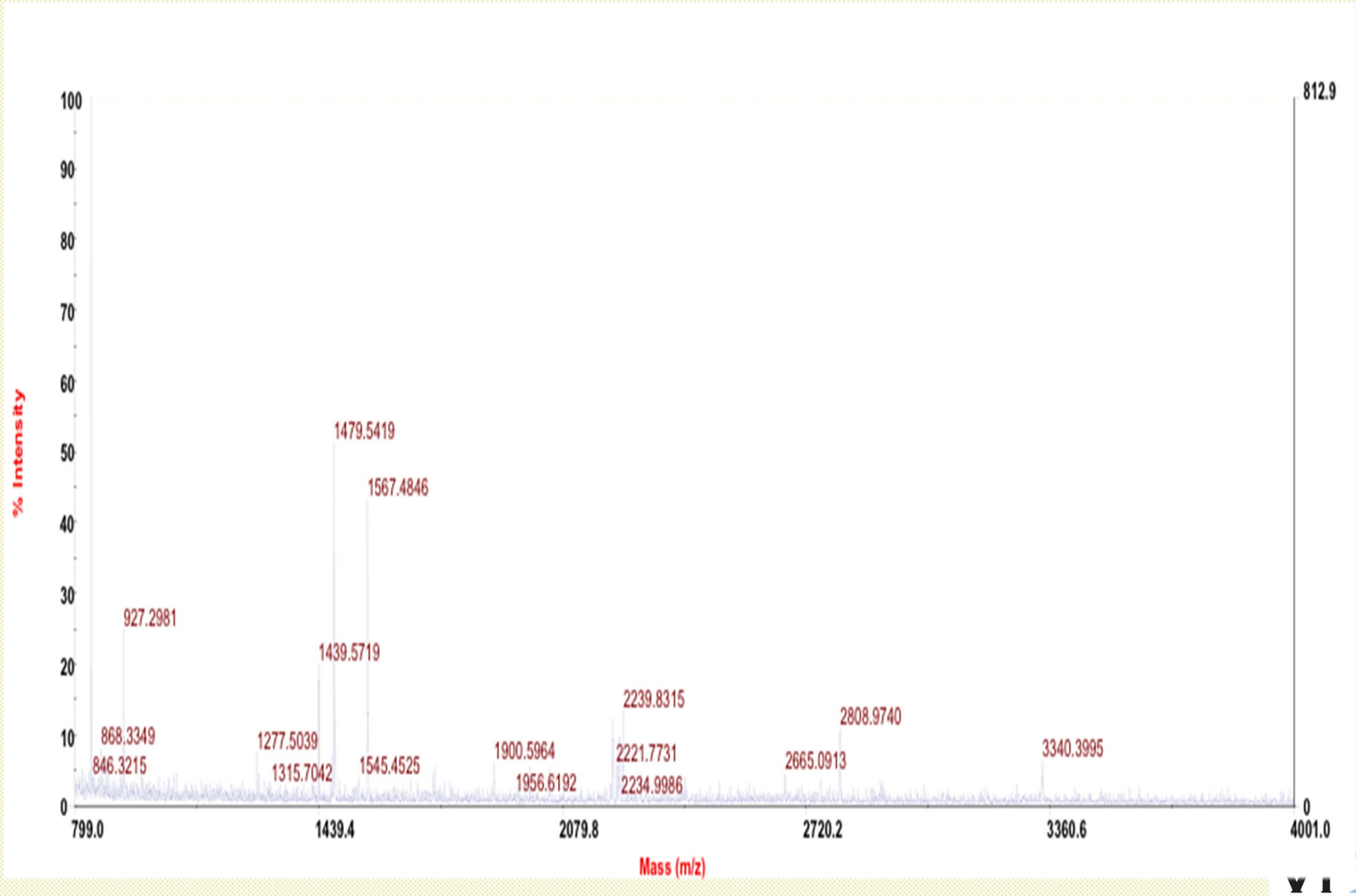


In-gel digestion à PMF detected by MALDI-TOF
stain: VisPro 5
75ng BSA
Mass fit research result: 44%



VISUAL PROTEIN

In-gel digestion à PMF detected by MALDI-TOF
stain: VisPro 5
25ng BSA
Mass fit research result: 27%



Recovery activity protein in gel

Q6 Recovery activity protein in gel: When recovery protein, we need to use CBR stain or other stain to check protein's position. But all commonly used stain cause protein loss activity. So we need a new stain can recovery protein and keep protein activity.

Comparison of other stain, VisPRO stain don't contain any acid compounds. It can't denature protein and make protein fixing in gel. Therefore, it can recovery the active enzyme from gel.

VisPRO Stain's compound doesn't combine with protein; therefore, VisPRO is the compatibility different applications.

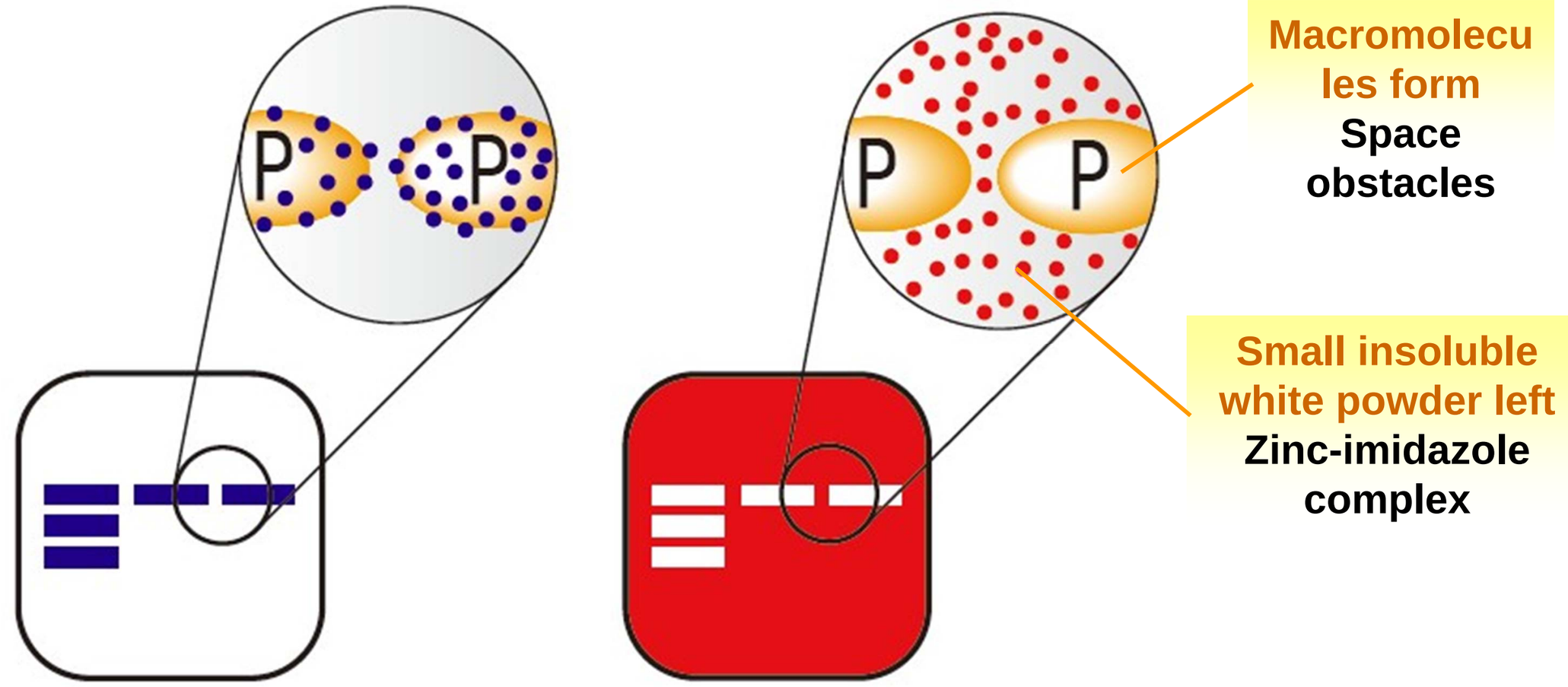
AL
PROTEIN

What’s Reverse Staining ?

(VisPRO 5 minutes Protein Stain Kit)

PostiveStain

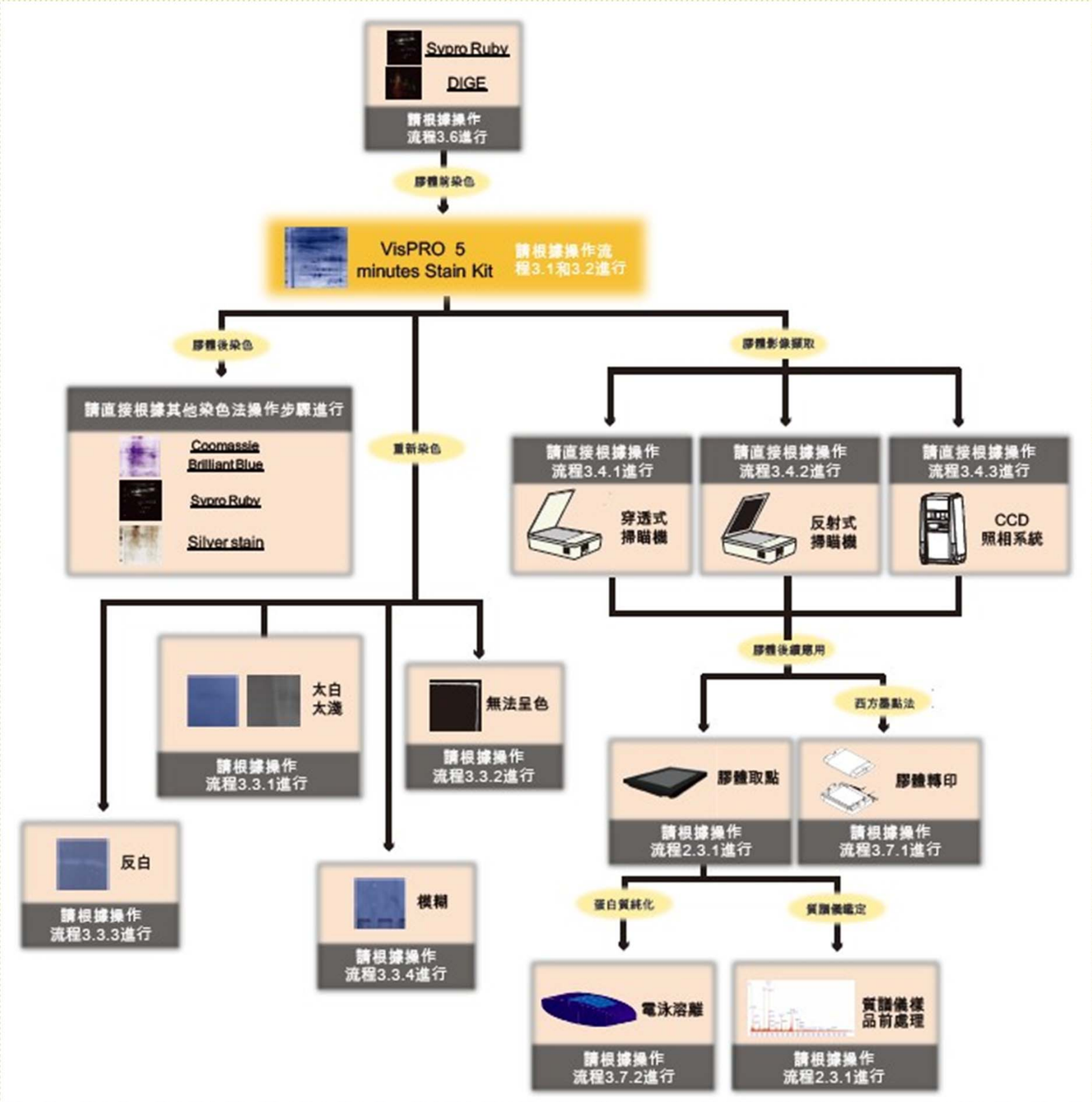
Reverse Stain



Reverse Stain: Stain non-protein part in gel and enhance the target protein by background

VISUAL PROTEIN

The compatibility to most applications with VisPRO Stain



1. Application other stain
2. MS identification
3. Blotting in WB
4. Electrophoresis elution
5. activity staining and detection

VISUAL PROTEIN

the method of the highest C/P stain

– **Q7 Expensive. To Consider the cost of reagents and the cost of time, VisPRO staining can obtain the higher C/P value.**

The cost of VisPRO Stain

To Consider the cost of reagents and the cost of time, VisPRO staining can obtain the higher C/P value.

VISUAL PROTEIN

Comparison of staining methods

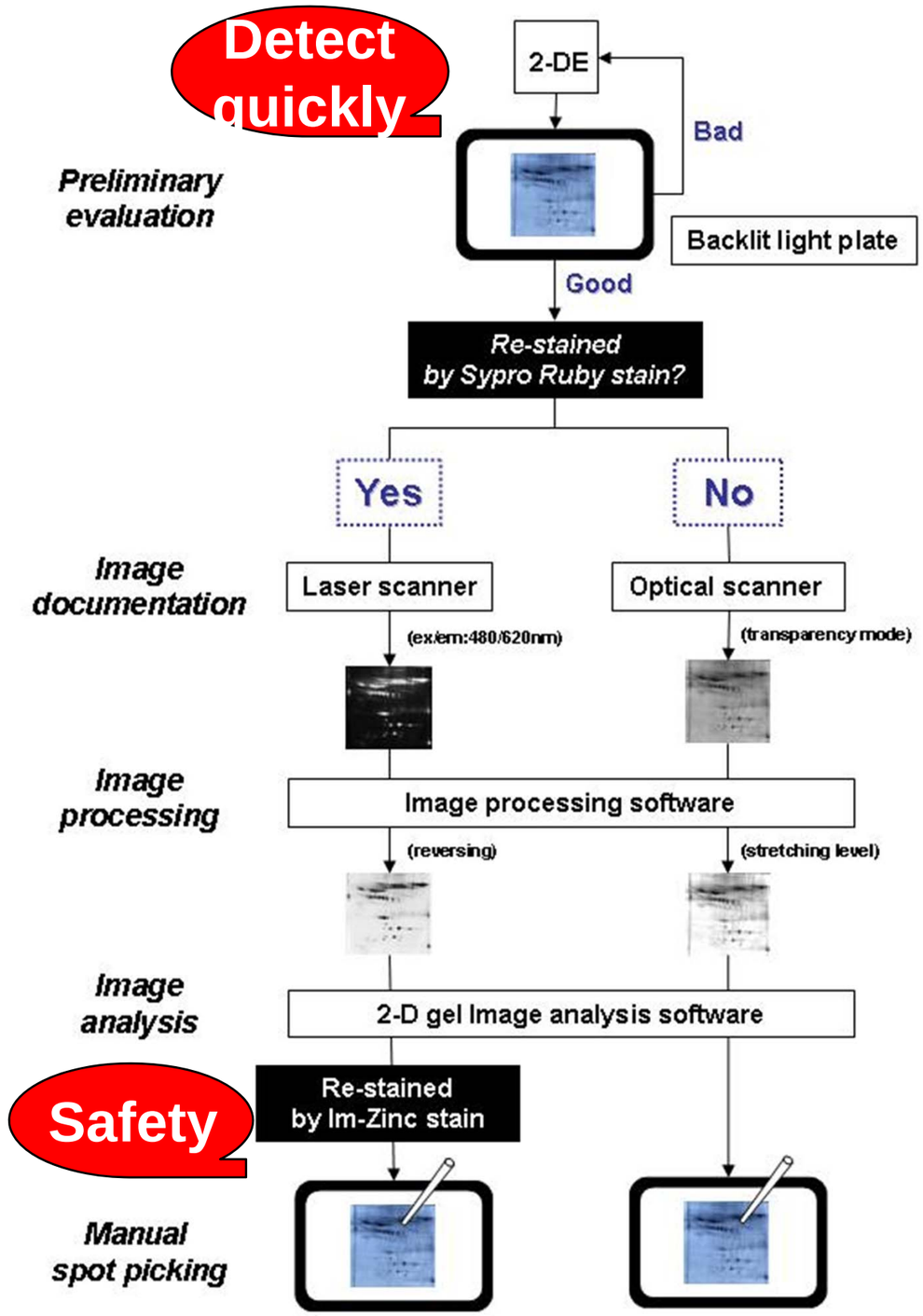
Methods	VisPRO Stain	Sypro™ Ruby	Silver Stain	CBR Stain
Total time	★ **5 ~15 min**	2.5~o/n	3.5~o/n	2.5 hr~ o/n
Imaging instrument	★ **visible light (cheap)**	fluorescent (expensive)	★ **visible light (cheap)**	★ **visible light (cheap)**
Irritant or toxic chemicals	★ **no**	Acetic acid, Methanol UV	Acetic 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	50-1000 ng
Capability to downstream applications	★ **Yes Compatibility with MASS and downstream applications**	★ **Yes Compatibility with MASS**	Limited protein cross link Low Compatibility with MASS	Limited Low compatibility with MASS

Safe and Easy to use

Q8 Using SYPRO Ruby, it is hurt for eyes while watching with UV.
It is invisible completely by cy dye.
It is inconvenient to select protein spot, either.
Is there any suggestion in safety and convenience?

Spending 5 minute only
Detect protein levels accurate to 1 ng with high sensitivity
None reaction with protein and MASS
SYPRO Ruby and Cy dye are complemented by VisPRO

VISUAL PROTEIN



[2D application]

VisPRO Stain combine with other Fluorescent stain

the visualized result with eyes

take the point correctly and position completely

without the risk of UV

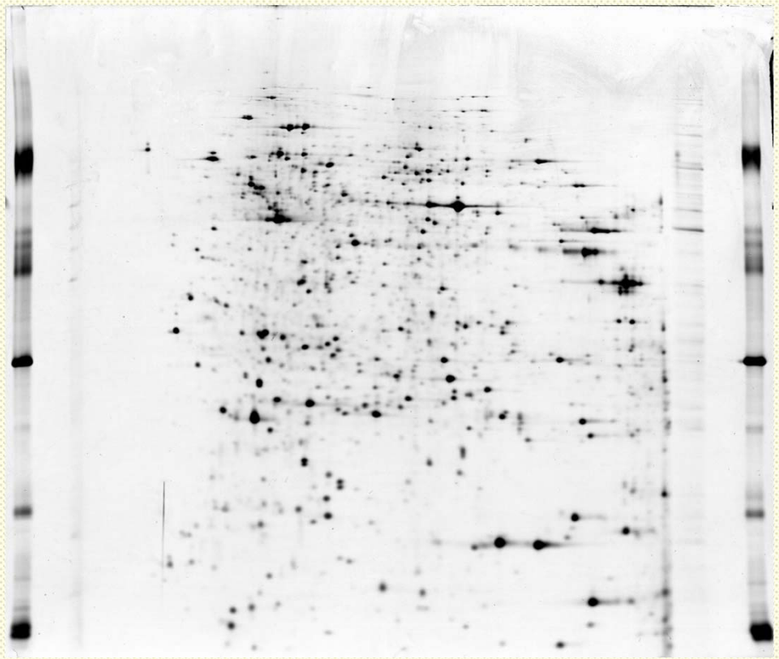
compatibility with MASS and downstream application

VISUAL PROTEIN

The result and observation: Reverse Staining (VisPRO 5 minutes Protein Stain Kit)

- After staining：
  - Gel stained milk-white color
  - Protein sample is color less

- Observing under the dark background
  - Clearly to observe the colorless sample band changes to black.



Staining result for 2-DE gels by VisPRO 5 minutes Protein Stain Kit

VISUAL PROTEIN

VisPRO 5 minutes Protein Stain kit
The three high features

❿ High Speed

❿Only 5~15 min staining process

❿ High Sensitivity

❿Detect protein levels accurate to 1ng

❿ High Compatibility

❿Apply to mass spectrometry analysis

❿Restain by other methods

❿Recycle proteins

❿Gels stained with VisPRO can be re-stained by other protein staining methods or WB.

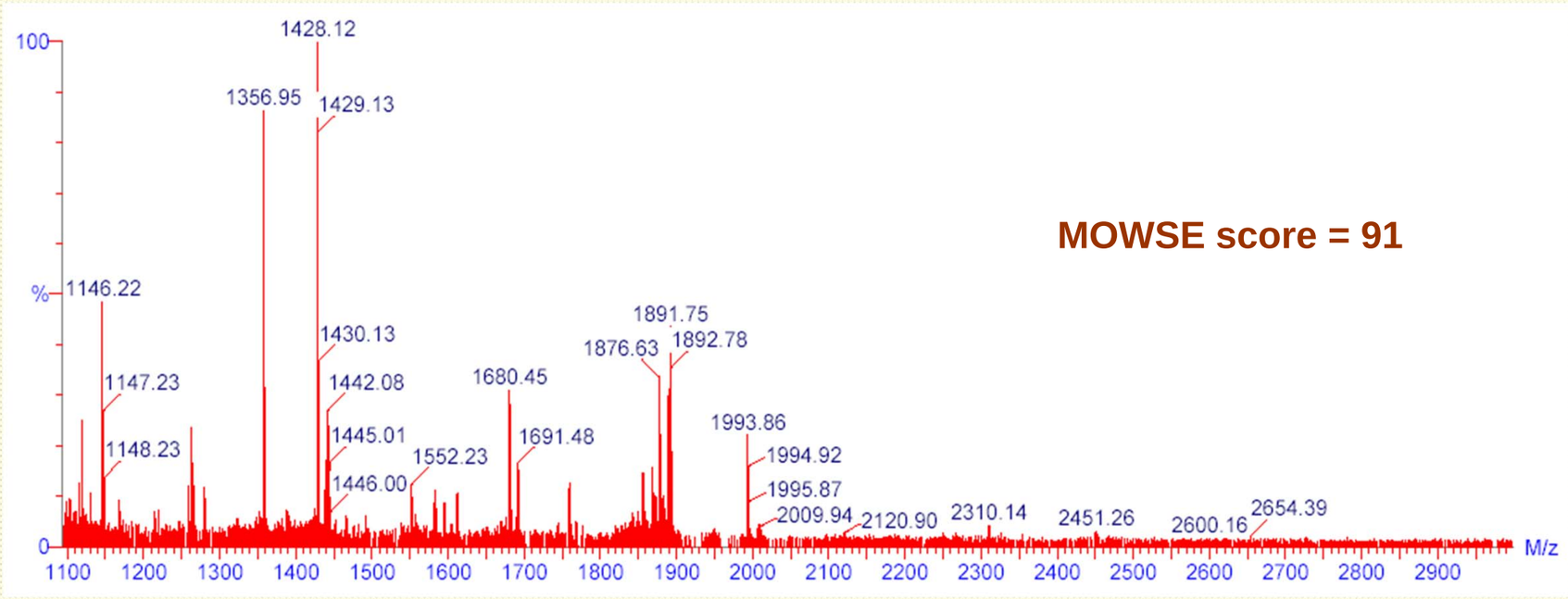
UAL PROTEIN

The features of VisPRO 5 minutes Protein Stain Kit

- **Safety**
  - Non-toxic
  - non- highly toxic heavy metal
  - Visualized with naked eye（without UV）
- **Convenience**
  - Stored at room temperature for two year before opening.
  - Use it without any preparation
- **Economy**
  - available price
  - Unlimited principle
  - Cheap image and accessories

VISUAL PROTEIN

Reverse Stain's (VisPRO's)
Capability to downstream applications—MASS analysis



MOWSE score = 91

The MALDI-TOF spectrum of spots from zinc reverse staining.

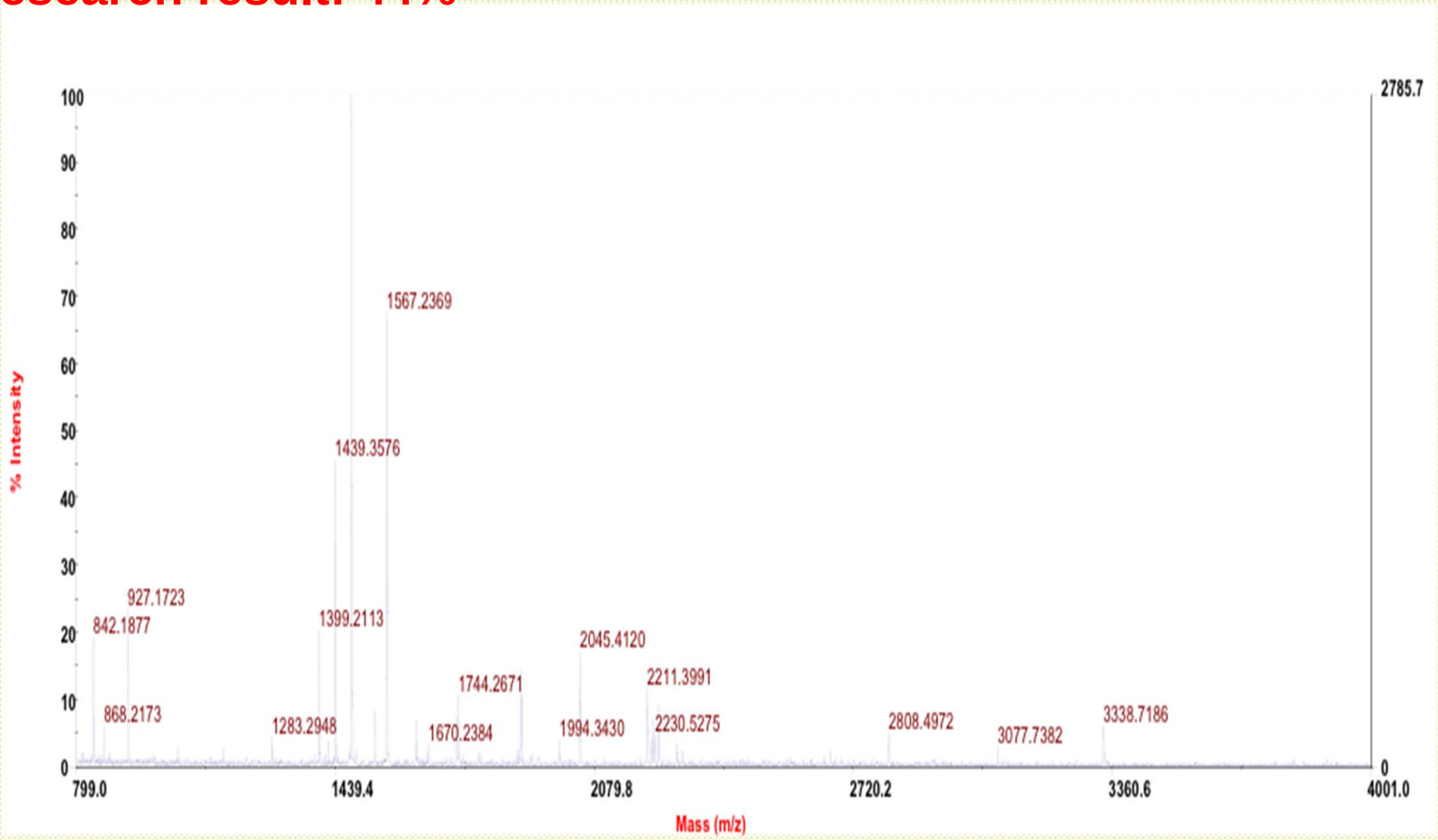
The zinc reverse staining spot of phosphorylase b was cut and subjected to mass sample preparation. The observed ionized fragments of rabbit phosphorylase b were annotated.

⑩Reverse stain is perfectly suitable with mass spectrometry analysis

JAL PROTEIN

Reverse Stain's (VisPRO's)
Capability to downstream applications—MASS analysis

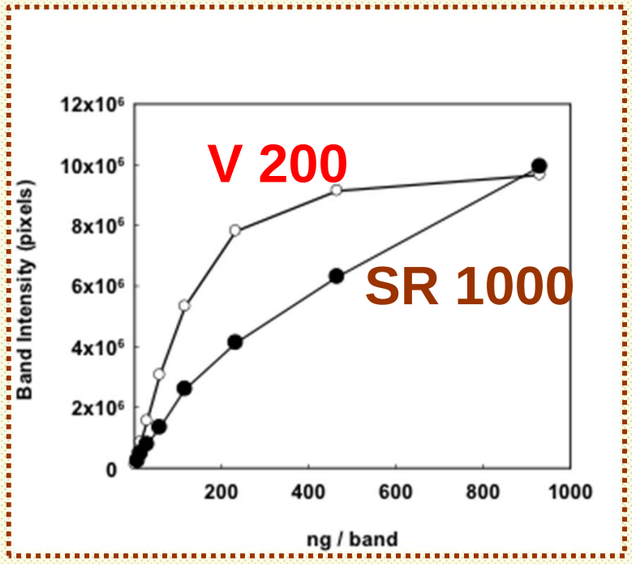
In-gel digestion à PMF detected by MALDI-TOF
stain: VisPro 5
75ng BSA
Mass fit research result: 44%



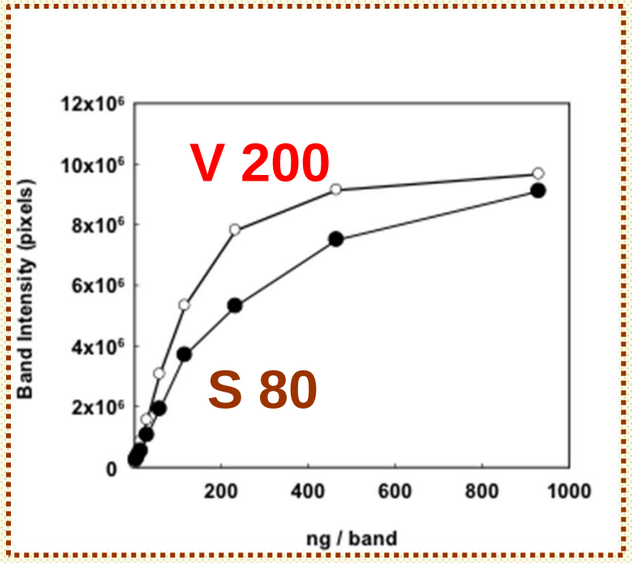
Reference：VisPRO is tested by the Instrument Center, Institute of Biomedical Sciences, Academia SINICA**, in July 2007**

VISUAL PROTEIN

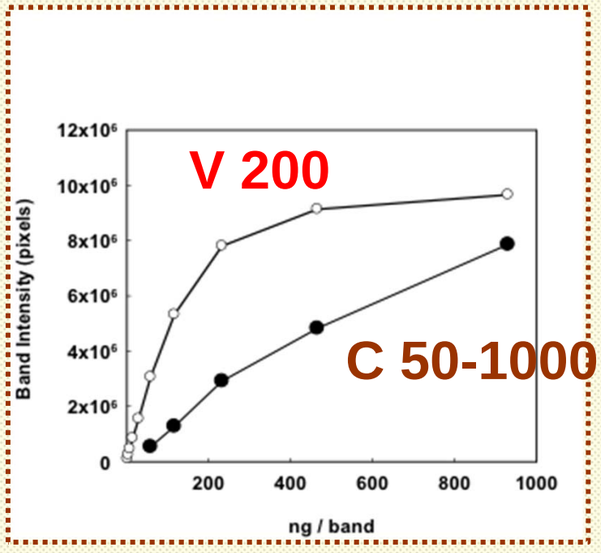
Comparison of linear range:
Reverse Stain (VisPRO) VS others



VisPRO vs. Sypro Ruby



VisPRO vs. Silver Stain



VisPRO vs. CBR Stain

VISUAL PROTEIN

Comparison of staining methods

Methods	Reverse Stain (VisPRO)	Sypro™ Ruby	Silver Stain	CBR Stain
Total time	★ **5 ~15 min**	2.5~o/n	3.5~o/n	2.5 hr~ o/n
Imaging instrument	★ **visible light （cheap）**	fluorescent （expensive）	★ **visible light （cheap）**	★ visible light **（cheap）**
Irritant of toxic chemicals	★ **no**	Acetic acid, Methanol UV	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	50-1000 ng
Compatibility to downstream application(e. g. WB & MASS)	★ **Yes High compatibility with MASS and other**	★ **Yes Compatibility with MASS**	Limited protein cross link low compatibility with MASS	Limited Low compatibility with MASS

Why is the reverse stain not popular before 2000?

⑩Image storage technology

- ⑩Zinc-imidazole reverse stain was created in 1990
- ⑩In 1990, adhesive save or silver photo preservation
- ⑩After the year of 2005, Digital photo or reflective scanner
- ⑩After the year of 2005, Visual Protein provides an see-through scanner, rising the quality and sensitivity on image.

⑩Equipment

- ⑩In 2005, Visual Protein provides **black staining box a t plate which can be easy visualized with naked eye**

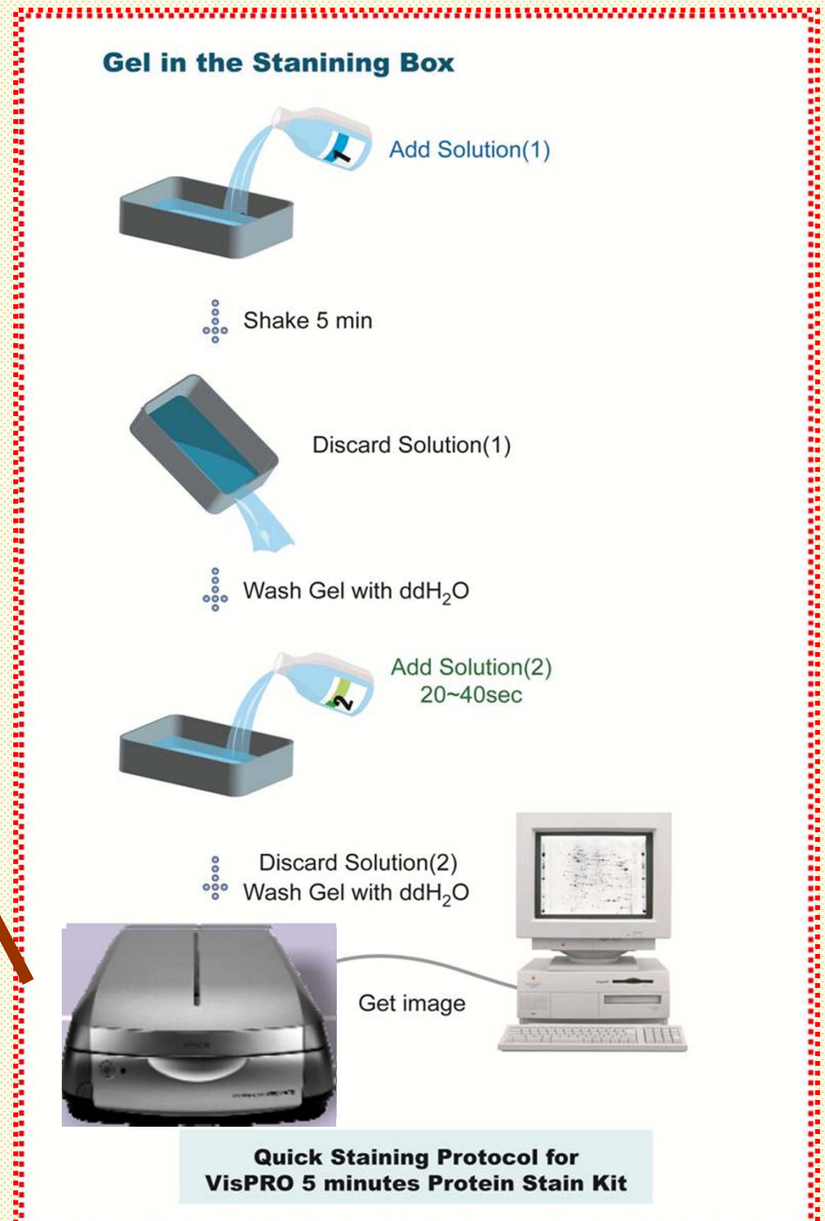
⑩ the Change of needs for the laboratory

- ⑩Enquiry for high excellent sensitivity
- ⑩Be **compatible with mass spectrometry**

VISUAL PROTEIN

Protocol for VisPRO 5 minutes Protein Stain

1. Using Black Staining Box



VisPRO 5 minutes Protein Stain Kit

2. Scan image See-through scanner EPSON V750 PRO

3. View and select spot by Gel Lighting Plate

VISUAL PROTEIN