Dr. Phillip Steindel

Review Editor, *JoVE*

1 Alewife Centre, Suite 200  
Cambridge, MA 02140  
Tel: 617.674.1888

3rd Aug.2018

MS ID:  **JoVE58669**

Subject: **Invited Video-Article**

MS Title: **Fluorescent Silver Staining of Proteins in Polyacrylamide Gels**

MS Authors: **Alex Wong, Sheng Xie, Ben Zhong Tang and Sijie Chen*\****

**Dear Dr. Phillip Steindel,**

Thank you very much for your email on 24 Jul. 2018 and for forwarding us the reviewers’ comments and suggestions. We have revised our manuscript according to all the comments. All the modifications are tracked in the revised manuscript. Given below are our specific point-by-point responses to the reviewers’ comments.

***Response to your comments***

1. *Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.*

The manuscript has been proofread to correct any spelling or grammar issues. The spelling has been made consistent with standard American English.

1. *Please expand the Summary (10-50 words total) to briefly describe the applications of this protocol.*

We have expanded the summary to briefly describe the applications of this protocol as requested: “Here, we describe a detailed protocol which outlines a new fluorescent staining technique for total protein detection in polyacrylamide gels. The protocol utilizes a silver ion specific fluorescence turn-on probe, which detects the Ag+ -protein complex. This technique makes use of the specific bio-affinity of silver ions to proteins, but eliminates certain limitations surrounding traditional silver stains.”

*3. Please spell out each abbreviation the first time it is used.*

Correction have been made for abbreviations to be spelt out for its first use such as; Sodium Dodecyl Sulphate-Polyacrylamide-Gel Electrophoresis (SDS-PAGE), mass spectrometry (MS), Ultra-violet (UV) and Lithium Dodecyl Sulphate (LDS).

*4. JoVE cannot publish manuscripts containing commercial language. This includes trademark symbols (™), registered symbols (®), and company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents. For example: Invitrogen, Thermos Fisher Scientific, Millipore, PowerEase, NUPAGE, Azurespot, etc.*

Thank you for the reminder, the trademark symbols and names have been removed from the manuscript and table of materials in the revision.

*5. Please revise the protocol text to avoid the use of any personal pronouns (e.g., "we", "you", "our" etc.).*

The personal pronouns have now been removed in the revised protocol. “The dye TPE-4TA was synthesized following the protocol that was recently reported.10”

*6. Please revise the protocol to contain only action items that direct the reader to do something (e.g., “Do this,” “Ensure that,” etc.). The actions should be described in the imperative tense in complete sentences wherever possible. Avoid usage of phrases such as “could be,” “should be,” and “would be” throughout the Protocol. Any text that cannot be written in the imperative tense may be added as a “Note.” Please include all safety procedures and use of hoods, etc. Please move the discussion about the protocol to the Discussion.*

We have edited the words to the suggested action items in the revision and removed certain phrases as mentioned above.

*7. Lines 84-91: Please revise this paragraph to discrete numbered steps.*

We have revised the brief description of conditions for sample preparation and electrophoresis into discrete numbered steps.

*8. In the JoVE Protocol format, “Notes” should be concise and used sparingly. They should only be used to provide extraneous details, optional steps, or recommendations that are not critical to a step. Any text that provides details about how to perform a particular step should either be included in the step itself or added as a sub-step. Please consider moving some of the notes about the protocol to the discussion section.*

We have revised the ‘Notes’ as suggested.

*9. 3.2: Please specify how to adjust the pH of the solution.*

The revision now specifies how to check and adjust the pH of the solution: “To prepare 100 mL of the fluorogenic developing solution (10 µM), add 10 mL of the **TPE-4TA** stock solution into 90 mL ultra-pure water. Check the pH of the solution using a pH meter and tune to 7-9 using acetic acid (1 mM) or sodium hydroxide (1 mM).”

*10. 3.3: Please specify the incubation temperature.*

The incubation temperature is at room temperature and is specified in the revision.

*11. 4.2: What volume of water is used to rinse? How many times?*

The gel is rinsed in 100 mL of water once, for a period of 5 min.

*12. 4.3: Please add more details about how to image the gel.*

The gel can be visualized with any UV lamp containing commercial gel documentation machine at the 302 nm and 365 nm channel. This has been specified more clearly in the revision.

*13. Discussion: Please also discuss critical steps within the protocol and any limitations of the technique.*

The critical step in the discussion includes the adjustment of pH and preventing the gel from acid exposure. Furthermore, an additional critical information within the protocol has been discussed, which is to follow the suggested silver nitrate concentration in the protocol as any higher may result in high background staining. Other limitations of the technique to be included in the revision is the fact that the dye is not widely commercially available and that the AIE development takes longer than a conventional chemical development.

*14. References: Please do not abbreviate journal titles. Please include volume and issue numbers for all references.*

The journal titles have now been corrected in the revision with the volume and issue numbers included.

*15. Please follow the book citation example below to reformat book references:  
Kioh, L.G. et al. Physical Treatment in Psychiatry. Blackwell Scientific Pubs. Boston (1988).*

Thank you for your suggestion, reference [6] now follows the example provided. Celis, J. E. et al. Cell Biology: A Laboratory Handbook. Elsevier Academic Press. Amsterdam (2006).

*16. Please remove trademark (™) and registered (®) symbols from the Table of Equipment and Materials.*

Thank you for the reminder, trademarks, symbols and commercial names have been removed from the Table of Equipment and Materials.

***Response to the Comments and Suggestions of Reviewer 1***

1. *data suggest that apparently Sypro can stain proteins better than Silver staining, which is not normally true, of course it depends on the quality of silver staining, for example if silver stain is conducted by a nice protocol and properly or for example with Blue silver (see Electrophoresis. 2004 May;25(9):1327-33, that should be at least cited along with and its advantages/disadvantages compared to the method proposed) it is definitely more sensitive. The only real advantage of the proposed method, apart from its being fluorescent, is that it's fast and intrinsically displaying very low background.*

We fully agree with the comment regarding the SYPRO stain and silver stain. There are many silver staining protocols. In this study we used a simple silver nitrate stain, which has minimal steps and can be further modified for the silver impregnation step of the fluorescent silver stain. This serves as a control and benchmark for our fluorogenic silver stain to see how much the AIE dye can improve the performance in terms of sensitivity and contrast, when combined with the silver ions. This allows direct observation of how much the performance can improve with the AIE fluorophore without any extraneous influence from other reagents.

There are numerous silver staining protocols to compare. In particularly when a sensitization step is applied, the detection of silver staining can be much improved. We also noticed the highly sensitive blue silver method, which is now cited in the revised version. Basically, we also prefer a silver nitrate staining protocol variant in the absence of an aldehyde sensitizer since it may lead to a stain compatible with mass spectroscopy.

1. *Another drawback is the need for a particular device for the reading, anyway this should not be a problem.*

Yes, this is true for many fluorescence-based techniques. In our case, it is simple because many gel imagers have UV channels and thus can be used directly to read the gel.

***Response to the Comments and Suggestions of Reviewer 2***

1. *The control method to which the new method has been compared, is not adequate. There are many other variants of silver staining that are one order of magnitude more sensitive than the one selected. It is as if someone describing a new staining method with a dye would compare it to old style Coomassie blue with alcohol-acid de-staining and not to modern colloidal Coomassie blue.*

Thank you for your comment. There are many silver staining protocols. In particular when a sensitization step is used, the quality can be improved highly. For the same protocol, the staining quality will also vary from operator to operator. There is no silver staining protocol that can be claimed as the best for use as a standard silver staining method for comparison. Regarding the sensitivity, our method cannot exceed the detection limit of the best silver stain in this sample (the reported LOD is lower than many of the paper reporting highly sensitive silver staining method). That is why the SYPRO method is also used in parallel as a control group. SYPRO is a widely used fluorescent stain, and its performance has been fully compared with many silver stains.

In this work, we want to highlight the use of “fluorogenic development”, instead of the classic chemical development which reduces the silver ions. Taking this into account, the control method we have selected is fair to compare. Furthermore this allows us to understand the difference in the performance between our novel method and the basic fundamental silver staining method. We selected this protocol variant of the silver stain, specifically because it uses silver nitrate and avoids many harmful chemicals such as ammonia and glutaraldehyde without compromising MS compatibility.

Taking this into account, our selected silver protocol is a suitable control regarding the design of our staining strategy. Many other silver stains, in particular the sensitive ones, can be good alternatives to compare the detection limits in a fair way. Meanwhile, we adopt the standard SYPRO fluorescent stain as a suitable control to discuss about the sensitivity in the detection of proteins.

1. *The organic probe which is absolutely mandatory for the method is not widely available. Most of the end users do not have the chemical knowledge to prepare it or the money to buy a custom synthesis.*

At the time of writing, the AIE probe we have described is not yet widely available, however the probe is patented and will be commercialized very soon.

Please let me know if further information is required.

 Yours sincerely,



Sijie Chen, Ph.D.

Assistant Professor

Karolinska Institutet