Sensitive silver staining

Development Technique File No. 210
PhastSystem

This technique file describes optimized silver staining methods for detecting proteins in PhastGel™ IEF gradient media. The methods are designed to give reproducible, highly sensitive staining with low, uniform background staining. Results are obtained within 60 to 75 minutes (depending on the separation technique).

The average sensitivity limit of this silver staining technique is estimated to be 1 to 5 ng protein per band for IEF and native PAGE, and 0.3 to 0.5 ng protein per band for SDS-PAGE using PhastGel separation media. This method is more sensitive than the Coomassie™ staining method described in Development Technique File 200: approximately 20 times more sensitive for IEF and native PAGE; and approximately 60 times more sensitive for SDS-PAGE.

Introduction

The silver staining technique presented in this file is derived from the method of Heukeshoven and Dernick (1). The method has been optimized for silver staining PhastGel IEF and gradient media. To compensate for the differing properties between IEF, native, and SDS-PAGE gels, three methods have been developed.

The staining strategy is essentially the same for the three methods, namely: fixing and removal of buffer ions, "sensitizing" the proteins in a glutardialdehyde solution, removal of excess glutardialdehyde, reaction with silver ions in a silver nitrate solution, developing in a basic formaldehyde solution and stopping the development in acetic acid. A final rinsing step in 10% acetic acid/5% glycerol is used to prevent gradient gels from curling or cracking after drying.

Little or no background staining occurs with PhastGel gradient media (except in the stacking gel zone). A uniform, transparent yellow background will develop with PhastGel IEF media. This is most likely due to the lower polyacrylamide concentration, since the same background staining occurs in the stacking gel zone of the PhastGel gradient media. This yellow background does not impair reading or scanning results. A clear background for IEF gels can be obtained by modifying the method, but only at the expense of sensitivity.

After development, the gels are dried. Dried gels are easily stored in notebooks, or cut and mounted in slide frames.

Materials and Methods

Prepare the following solutions as described for PhastGel IEF and gradient media below. A volume of 80 ml is required to fill the chamber. The solutions are listed in order to use. Label all bottles with their corresponding in-port number to eliminate confusion later when connecting the bottles to the ports.

Prepare all solutions in the purest (deionized or double distilled) water available. We use Milli-Q™ water which has approximately 1 µohn conductivity levels.

1. Fixing solution: 20% trichloroacetic acid in water. For IEF and native PAGE gels only.
2. Washing: 50% ethanol and 10% acetic acid in water.
3. Washing: 10% ethanol and 5% acetic acid in water.
4. Sensitizing: 8.3% glutardialdehyde in water. Prepare from 1 part 25% commercially available solution and 2 parts water.
5. Washing: Deionized water or similar water with low conductivity. Have at least 320 ml in a bottle for the wash steps in the method.
6. Silver solution: 0.25% (w/v) silver nitrate in water for SDS-PAGE gels.

   Silver solution: 0.5% (w/v) silver nitrate in water for IEF and native PAGE gels.

   Ensure that the silver nitrate dissolves completely for reproducible results.

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7. Developing solution: 0.015% formaldehyde in 2.5% sodium carbonate. Prepare a 12.5% (w/v) stock solution of sodium carbonate in water. For a 1 liter solution, prepare the developer as follows: 200 ml Na_2CO_3 (12.5%) + 800 ml H_2O + 400 µl of a 37% aqeous solution of formaldehyde. Prepare this solutions fresh the day you plan to use it.
8. Stop solution: 5% acetic acid in water.
9. Preserving solution: 10% acetic acid and X% glycerol in water. Not necessary for IEF gels.

Note: the concentration of glycerol is different for different gels. 2.5% for PhastGel homogeneous 7.5, 5% for PhastGel 10–15 and 12.5, 10% for PhastGel 8–25 and 20.

Use these solutions only once: Do not recycle. Except for the developing solution the solutions are stable for 2 to 3 days at ambient temperature (20–25 °C). The developer must be prepared fresh the day you plan to use it.

Notes: Since silver staining is a very sensitive method, do not touch the gel surface (before or after development). Use gloves when handling the buffer strips. If possible, filter or centrifuge your samples.

PhastGel IEF media

Program the method in table 1 into the development method file for silver stainig PhastGel IEF 3–9, 5–8, and 4–6.5. This method is valid for electrophoretic titration curves using these media.

PhastGel electrophoresis media

Program the method in table 2 onto the development method file for silver staining after SDS-PAGE.

Program the method in table 3 into the development method file for silver staining after native PAGE.

Procedure

The procedure for programming and running the development methods in tables 1, 2, and 3 is the same for both PhastGel IEF and electrophoresis media: program the methods(s), connect the solutions to the correct ports, insert the gel(s), close the lid and press the start button. Full instructions are given in the chapter on Operation in the PhastSystem™ Users Manual.

Checklist

Before starting development methods, you should run through the following checklist to avoid making mistakes:

1. Are the bottles connected correctly?
2. Are the tubes securely submerged in the solutions? Also, check for kinks or obstructions in the tubing.
3. If 2 gels are developed simultaneously, are the gels inserted so that their surfaces face one another?
4. If 1 gel is developed, is it in the lower position of the gel holder, and is the gel surface facing upwards?
5. Is the lid closed tightly?
6. Have you chosen the correct method from the development file?

Table 1. Silver staining method optimized for PhastGel IEF media to program into the development method file.

Step number	Solution[1]	IN-port	OUT-port[2]	Time	Temperature
1	20% TCA	1	0	5 min	20 °C
2	50% ethanol, 10% HAc	2	0	2 min	50 °C
3	10% ethanol, 5% HAc	3	0	2 min	50 °C
4	10% ethanol, 5% HAc	3	0	4 min	50 °C
5	8.3% glutardialdehyde	4	0	6 min	50 °C
6	10% ethanol, 5% HAc	3	0	3 min	50 °C
7	10% ethanol, 5% HAc	3	0	5 min	50 °C
8	Milli-Q water	5	0	2 min	50 °C
9	Milli-Q water	5	0	2 min	50 °C
10	0.5% silver nitrate	6	0	10 min	40 °C
11	Milli-Q water	5	0	0.5 min	30 °C
12	Milli-Q water	5	0	0.5 min	30 °C
13	Developer	7	0	0.5 min	30 °C
14	Developer	7	0	4 min	30 °C
15	5% HAc	8	0	5 min	50 °C

[1] Abbreviations used are:
TCA = Trichloroacetic acid; HAc = Acetic acid. All the solutions are made with water (Milli-Q or deionized).
[2] None of the solutions should be recycled.

Table 2. Silver staining method optimized for SDS-PAGE with PhastGel electrophoresis media to program into the development method file.

Step number	Solution[1]	IN-port	OUT-port[2]	Time	Temperature
1	50% ethanol, 10% HAc	2	0	2 min	50 °C
2	8.3% glutardialdehyde	4	0	6 min	50 °C
3	Milli-Q water	5	0	2 min	50 °C
4	Milli-Q water	5	0	2 min	50 °C
5	0.25% silver nitrate	6	0	13 min	50 °C
6	Milli-Q water	5	0	0.5 min	50 °C
7	Milli-Q water	5	0	0.5 min	30 °C
8	Developer	7	0	0.5 min	30 °C
9	Developer	7	0	4 min	30 °C
10	5% HAc	8	0	2 min	50 °C
11	10% HAc, X % glycerol	9	0	3 min	50 °C

[1] Abbreviations used are:
TCA = Trichloroacetic acid; HAc = Acetic acid. All the solutions are made with water (Milli-Q or deionized).
[2] None of the solutions should be recycled.

Table 3. Silver staining method optimized for SDS-PAGE with PhastGel electrophoresis media to program into the development method file.

Step number	Solution[1]	IN-port	OUT-port[2]	Time	Temperature
1	20% TCA	1	0	5 min	20 °C
2	50% ethanol, 10% HAc	2	0	2 min	50 °C
3	10% ethanol, 5% HAc	3	0	2 min	50 °C
4	10% ethanol, 5% HAc	3	0	4 min	50 °C
5	8.3% glutardialdehyde	4	0	6 min	50 °C
6	10% ethanol, 5% HAc	3	0	3 min	50 °C
7	10% ethanol, 5% HAc	3	0	5 min	50 °C
8	Milli-Q water	5	0	2 min	50 °C
9	Milli-Q water	5	0	2 min	50 °C
10	0.5% silver nitrate	6	0	10 min	40 °C
11	Milli-Q water	5	0	0.5 min	30 °C
12	Milli-Q water	5	0	0.5 min	30 °C
13	Developer	7	0	1 min	30 °C
14	Developer	7	0	5 min	30 °C
15	5% HAc	8	0	2 min	50 °C
16	10 HAc, X% glycerol	9	0	3 min	50 °C

[1] Abbreviations used are:
TCA = Trichloroacetic acid; HAc = Acetic acid. All the solutions are made with water (Milli-Q or deionized).
[2] None of the solutions should be recycled.

Sensitivity

The sensitivity of this technique was estimated using serial dilutions of the Calibration Kit proteins (for isoelectric point (pI) and molecular weight (MW) measurements). Examples are shown in Figs. 1 and 2.

The sensitivity will depend on the protein, since different proteins bind different amounts of silver. The sensitivity will also depend on the separation technique used. The sensitivity limit of this technique is expressed as an average for the proteins studied for PhastGel IEF and gradient media using the sample applicator 8/1 (8 lanes, each 1 µl). The bands are approximately 3 mm wide with this applicator.

The limit for IEF and native PAGE is 1 to 5 ng protein/band. This is approximately 20 times more sensitive than the Coomassie technique in development technique file number 200.

The limit for SDS-PAGE is 0.3 to 0.5 ng protein/band. This is approximately 60 times more sensitive than the Coomassie technique in development technique file number 200.

Reference

1. Simplified method for silver staining of proteins in polyacrylamide gels and the mechanism of silver staining. *Electrophoresis, 6 (1985) 1003–112*, Heukeshoven, J., Dernick, R.

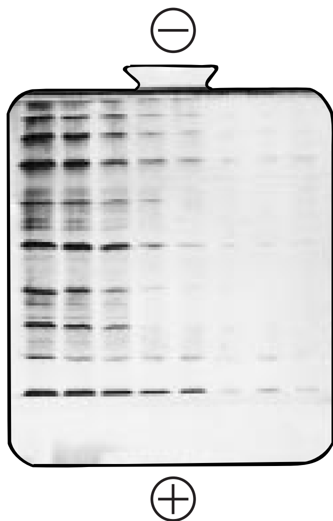

Fig. 1. Serial dilution of the Broad pI Calibration Kit on PhastGel IEF 3–9. Each vial in the kit contains approximately 20 µg of each of the proteins listed below. The dilution volume/vial is, starting from the left: 800 µl, 1.2 ml, 1.6 ml, 2.4 ml, 3.2 ml, 4.8 ml, 6.4 ml and 7.6 ml. The samples were applied in 1 µl volumes to each of 8 lanes.

From cathode to anode, the proteins are:
- lentil lectin (basic)
- lentil lectin (middle)
- lentil lectin (acidic)
- horse myoglobin (basic)
- horse myoglobin (acidic)
- human carbonic anhydrase B
- βovine carbonic anhydrase B
- b-lactoglobulin A
- soybean trypsin inhibitor
- amyloglucosidase

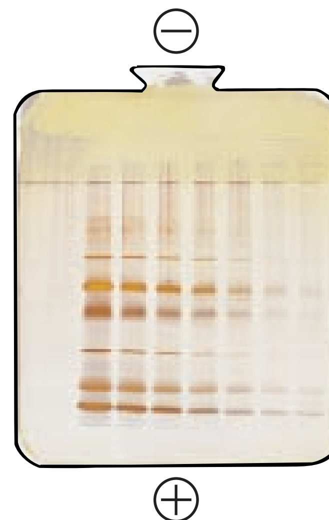

Fig. 2. Serial dilution of the LMW Calibration Kit (denatured) on PhastGel gradient 10–15 with SDS buffer strips. Each vial contains approximately 100 µg of each of the proteins listed below. The dilution volume/vial is, starting from the left: 1.6 ml, 3.2 ml, 6.4 ml, 12.8 ml, 25.6 ml, 52 ml and 102 ml. The samples were applied in 1 µl volumes to each of 7 lanes (the first lane is empty).

From cathode to anode, the proteins are:
- phosphorylase b
- albumin
- ovalbumin
- carbonic anhydrase
- trypsin inhibitor
- α-lactalbumin

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Produced by Wikströms, Sweden 980895, Aug., 1998
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