PhastGel™ Silver Kit Instruction Manual

PhastGel™ Silver Kit (Cat. No. 17-0617-01) is designed for fast, easy, and reproducible silver staining of PhastGel media with PhastSystem™.

PhastSystem revolutionized electrophoresis methodology and development. Fast, high-resolution, reproducible results are now easily obtained with PhastSystem. PhastGel Silver Kit brings these same benefits to the silver staining procedure. Ready-to-use solutions eliminate most of the mixing and handling of reagents that takes time and can affect reproducibility.

The protocols supplied with PhastGel Silver Kit are based on the procedures of Heukeshoven and Dernick (1 and 5) and Blum, Beier, and Gross (2). A new modification from the silver staining protocol supplied in the *PhastSystem Owner's Manual* involves the use of sodium thiosulphate to decrease non-specific background staining. This use of sodium thiosulphate results in gels with essentially clear backgrounds — making the gels much easier to interpret.

The sensitivity limits using this method with IEF and native-PAGE separations are 1 to 5 ng protein per band with standard proteins. The senstivity limit for SDS-PAGE separations of standard proteins is 0.3 to 0.5 ng protein per band. Alternatively, the use of glutaraldehyde with thiosulfate, alcohol and sodium acetate buffer results in a considerable increase in sensitivity, 0.05-0.1 ng protein per band (5). In comparison, coomassie blue procedures typically have sensitivities ranging from 5 ng protein per band to 100 ng protein per band (3).

Components of PhastGel Silver Kit

PhastGel Silver Kit includes reagents necessary for 10 runs (a total of 10 to 20 gels) in the Development Chamber of PhastSystem. The components of the kit are:

5x 150 ml bottles	5% glutaraldehyde
5x 150 ml bottles	0.4% silver nitrate
10x 150 ml bottles	2.5% sodium carbonate
10x 1.0 ml ampules	2.0% formaldehyde
10x 2.5 g packets	sodium thiosulphate

The researcher needs to provide ethanol (at least 95% ethanol, 5% isopropanol), glacial acetic acid, trichloroacetic acid, glycerol, Tris•HCl and reagent grade water.

Note: The chemicals used in this kit should not be discarded via public waste water systems. Please dispose of these chemicals properly. Please consult local regulations for information on proper disposal.

Instructions for Use of PhastGel Silver Kit

Program the staining protocol for your application into the Separation and Control Unit of PhastSystem. Tables 1, 2, and 3 give the steps for silver staining IEF, SDS-PAGE, and native-PAGE gels respectively.

Note: There are some differences in these protocols from the development programs in "Development Technique File No. 210" of the *PhastSystem Owner's Manual.* The protocols presented here have been developed after the ones listed in the *Owner's Manual* and can be viewed as improvements.

Table 1: Silver staining method optimized for PhastGel IEF media and titration curve analysis to be programmed into the development method file.

Step Number	Solution	IN	OUT	Time (min)	Temp (°C)	Remarks
1	20% TCA	1	0	5	20	Fixing solution
2	10% ethanol, 5% HAc	3	0	2	50	Wash solution
3	10% ethanol, 5% HAc	3	0	4	50	Wash solution
4	5% glutaraldehyde	4	0	6	50	Protein sensitization
5	10% ethanol, 5% HAc	3	0	3	50	Wash solution
6	10% ethanol, 5% HAc	3	0	5	50	Wash solution
7	Reagent grade water	5	0	2	50	Wash solution
8	Reagent grade water	5	0	2	50	Wash solution
9	0.4% silver nitrate	6	0	10	40	Staining solution
10	Reagent grade water	5	0	0.5	30	Wash solution
11	Reagent grade water	5	0	0.5	30	Wash solution
12	Developer	7	0	0.5	30	Developing solution
13	Developer	7	0	3.5*	30	Developing solution
14	Background Reducer	8	0	1.5*	30	Background reducing solution
15	Reagent grade water	5	0	5	50	Stop solution

Abbreviations used: TCA = trichloroacetic acid; HAc = acetic acid.
*These times are variable and are listed here to serve as a guideline. See the Optimization of Results section for details.

Table 2: Silver staining method optimized for SDS-PAGE with PhastGel gradient media to be programmed into the development method file.

Step Number	Solution	IN	OUT	Time (min)	Temp (°C)	Remarks
1	10% ethanol, 5% HAc	3	0	2	50	Wash solution
2	10% ethanol, 5% HAc	3	0	4	50	Wash solution
3	5% glutaraldehyde	4	0	6	50	Protein sensitization
4	10% ethanol, 5% HAc	3	0	3	50	Wash solution
5	10% ethanol, 5% HAc	3	0	5	50	Wash solution
6	Reagent grade water	5	0	2	50	Wash solution
7	Reagent grade water	5	0	2	50	Wash solution
8	0.4% silver nitrate	6	0	6.5	40	Staining solution
9	Reagent grade water	5	0	0.5	30	Wash solution
10	Reagent grade water	5	0	0.5	30	Wash solution
11	Developer	7	0	0.5	30	Developing solution
12	Developer	7	0	4.0*	30	Developing solution
13	Background Reducer	8	0	2.0*	30	Background reducing solution
14	5-10% glycerol	9	0	5*	50	Stop solution

Abbreviations used: TCA = trichloroacetic acid; HAc = acetic acid.
*These times are variable and are listed here to serve as a guideline. See the Optimization of Results section for details.

Table 3: Silver staining method optimized for native-PAGE with PhastGel gradient media to be programmed into the development method file.

Step Number	Solution	IN	OUT	Time (min)	Temp (°C)	Remarks
1	20% TCA	1	0	5	20	Fixing solution
2	50% ethanol, 10% HAc	2	0	2	50	Wash solution
3	10% ethanol, 5% HAc	3	0	2	50	Wash solution
4	10% ethanol, 5% HAc	3	0	4	50	Wash solution
5	5% glutaraldehyde	4	0	6	50	Protein sensitization
6	10% ethanol, 5% HAc	3	0	3	50	Wash solution
7	10% ethanol, 5% HAc	3	0	5	50	Wash solution
8	Reagent grade water	5	0	2	50	Wash solution
9	Reagent grade water	5	0	2	50	Wash solution
10	0.4% silver nitrate	6	0	10	40	Staining solution
11	Reagent grade water	5	0	0.5	30	Wash solution
12	Reagent grade water	5	0	0.5	30	Wash solution
13	Developer	7	0	1	30	Developing solution
14	Developer	7	0	5.0*	30	Developing solution
15	Background Reducer	8	0	2.0*	30	Background reducing solution
16	5-10% glycerol	9	0	5*	50	Stop solution

Abbreviations used: TCA = trichloroacetic acid; HAc = acetic acid.
*These times are variable and are listed here to serve as a guideline. See the Optimization of Results section for details.

Table 4: Modified high sensitivity silver staining method for SDS-PAGE PhastGel media(5) to be programmed into the development method file.

Step Number	Solution	IN	OUT	Time (min)	Temp (°C)	Remarks
1	10% ethanol, 5% HAc	3	0	2	50	Wash solution
2	10% ethanol, 5% HAc	3	0	4	50	Wash solution
3	"Fixing Buffer"	4	0	6	50	High sensitivity fix solution
4	10% ethanol, 5% HAc	3	0	3	50	Wash solution
5	10% ethanol, 5% HAc	3	0	5	50	Wash solution
6	Reagent grade water	5	0	2	50	Wash solution
7	Reagent grade water	5	0	2	50	Wash solution
8	0.4% silver nitrate	6	0	6.5	40	Staining solution
9	Reagent grade water	5	0	0.5	30	Wash solution
10	Reagent grade water	5	0	0.5	30	Wash solution
11	Developer	7	0	0.5	30	Developing solution
12	Developer	7	0	4.0*	30	Developing solution
13	Background Reducer	8	0	2.0*	30	Background reducing solution
14	5-10% glycerol	9	0	5*	50	Stop solution

Abbreviation used: HAc = acetic acid.
*These times are variable and are listed here to serve as a guideline. See the Optimization of Results section for details.

Preparation of Solutions

The following solutions should be prepared fresh on the day of use. When calculating volumes, remember that PhastSystem uses 75 ml of solution per step.

1. 20% trichloroacetic acid. This solution is used for all techniques except SDS-PAGE. Prepare 75 ml for 1 run.

   Caution: Can cause burns. Follow manufacturer's directions for handling.

2. 50% ethanol, 10% acetic acid. This is only used for native-PAGE gels.

3. 10% ethanol, 5% acetic acid. Prepare 300 ml for 1 run.

4. Developer. Tap the formaldehyde ampule gently on the bench top to remove any liquid from the neck of the ampule. Then use a pasteur pipette to transfer the contents of one ampule of 2% formaldehyde into one bottle of 2.5% sodium carbonate. Shake vigorously for 5 seconds.

   Caution: 2% formaldehyde can irritate skin, eyes, nose, and throat. Avoid breathing vapor. Wash hands after handling. 2.5% sodium carbonate may cause skin irritation.

5. Background Reducer. Add one packet of sodium thiosulphate (2.5 g) and 3.7 g Tris•HCl to 100 ml reagent grade water. Alternatively, 3-7 ml of glacial acetic acid may be added in place of Tris•HCl to bring the pH to between 5 and 6 prior to use.

6. "Fixing Buffer" as used in Step 3 of Table 4. Combine 10ml of 5% glutaraldehyde, 4ml of 2.5% sodium thiosulfate in water, 30ml of ethanol (95% ethanol, 5% isopropanol) and 0.3g of sodium acetate. Make up to 100ml with reagent grade water.

The 5% glutaraldehyde and 0.4% silver nitrate solutions are ready to use. No further preparation is required. Each bottle of these solutions provides the amount necessary for 2 development runs.

Caution: 5% glutaraldehyde can cause burns. Do not allow to come in contact with eyes, skin or clothing. Avoid breathing vapor. Wash hands thoroughly after handling. 0.4% silver nitrate may cause eye irritation and can stain skin and clothing.

After the staining solutions are prepared, begin the separation procedure. Connect the staining solutions to the Development Unit according to the appropriate protocol. Following completion of the separation, transfer the gel(s) to the Development Chamber and begin the staining procedure.

When the silver staining is completed, cap the silver nitrate and glutaraldehyde solutions and store at +4°C.

Optimization of Results

1. The Developer and Background Reducer step times can be increased or decreased to optimize the development and background levels for each specific protein. Each solution can affect both the intensity of the protein band and the background of the gel. A balance must be struck between the two solutions to obtain a gel with dark bands and a clear background.

2. Use fresh solutions for the silver staining. Solutions that are several days old will not give optimal results.

3. Make sure that all tubing reaches the bottom of the solution bottles.

4. Handle undeveloped gels with gloves; silver staining will show fingerprints.

5. Gently swab the gel surface after development to remove any excess silver that may be deposited on the gel.

6. Dry the gels as soon as possible after staining is complete. Do not let the gels stay in the Development Chamber for an extended period of time.

7. Go through a cleaning procedure for the tubing and Development Chamber at the end of each day. See pages 32-33 in the "System Guide", section 2 of the *PhastSystem Owner's Manual* for a protocol.

8. Use a cotton swab to gently clean the temperature probe and level sensor in the Development Chamber.

9. Rinse the Development Chamber gasket periodically to remove build-up of staining reagents.

10. Change the solution tubing at regular intervals.

11. The percentage of glycerol required is dependent upon the type of PhastGel media used.

References

1. Simplified method for silver staining of proteins in polyacrylamide gels and the mechanism of silver staining. *Electrophoresis 6 (1985) 103-112.* Heukeshoven, J. and Dernick, R.

2. Improved silver staining of plant proteins, RNA, and DNA in polyacrylamide gels. *Electrophoresis 8 (1987) 93-99.* Blum, H., Beier, H., and Gross, H.J.

3. Clear background and highly sensitive protein staining with Coomassie Blue dyes in polyacrylamide gels: a systematic analysis. *Electrophoresis 6 (1985) 427-448.* Neuhoff, V. *et. al.*

4. *PhastSystem Owner's Manual (1986).* Pharmacia AB, Uppsala, Sweden.

5. Improved silver staining procedure for fast staining in PhastSystem Development Unit I. Staining of sodium dodecyl sulfate gels. *Electrophoresis 9 (1988) 28-33.* Heukeshoven, J. and Dernick, R.

Amersham Biosciences
Uppsala, Sweden

Amersham Bioscience
Piscataway, N.J. 08855

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