UVP

Application Bulletin

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STAINING PROTOCOLS

UVP has been a leading manufacturer of high quality instrumentation for the detection of nucleic acids and proteins. Our wide selection of transilluminators and epi-illuminators complements the ever expanding range of stains and dyes on the market today. We offer transilluminators in various wavelengths, in the absorption spectra of popular fluorescent stains such as ethidium bromide, SYBR Green, SYBR Gold, SYPRO orange, and GelStar. The white light transilluminator is useful when viewing protein dyes such as bromophenol blue and silver stain. We have included short protocols of the stains and dyes mentioned above for quick reference. For more details please refer to the web pages listed on the bottom of this bulletin.

NUCLEIC ACID DETECTION IN GELS

ETHIDIUM BROMIDE

EX/EM: 518/605

Ethidium bromide can be used to stain the gel after electrophoresis or be incorporated into the gel and buffer before electrophoresis. Higher resolution can be obtained if the gel is stained after running electrophoresis. Ethidium bromide is a mutagen and should be handled with care.

PRE-STAINING WITH ETHIDIUM BROMIDE

Prepare agarose solution, add 0.5µg of ethidium bromide to 1ml of the cooling solution. Cast the gel. Add 0.5µg of ethidium bromide to 1ml of running buffer. Load and run the gel. Submerge the gel in distilled water for twenty minutes and again in fresh distilled water to destain it. View the gel with an UV transilluminator or hand-held lamps.

POST-STAINING WITH ETHIDIUM BROMIDE

Prepare enough ethidium bromide solution, 0.5µg to one ml of distilled water or running buffer. Submerge the gel, after electrophoresis, in the solution for twenty minutes. Submerge the gel in distilled water for twenty minutes. Repeat with fresh distilled water. View the gel with an UV transilluminator or hand-held lamps.

SYBR® GREEN I

EX/EM: 494/513

SYBR Green I is more sensitive then ethidium bromide and less mutagenic. Like ethidium bromide, SYBR Green I can be used after electrophoresis or incorporated into the gel before electrophoresis. Staining the gel after electrophoresis results in higher resolution of the bands. Incorporating the stain in the gel can induce the gel to be more sensitive to DNA overloading.

PRE-STAINING WITH SYBR GREEN I

Thaw the SYBR Green I solution. Spin solution in a microcentrifuge. Add SYBR Green I to the cooling agarose solution to a concentration of 1ml of SYBR Green I to 10000ml of gel solution. Cast the gel. Perform electrophoresis. View the gel using a 300 nm UV transilluminator or 254 nm epi-illuminator. There is no need for destaining.

POST-STAINING WITH SYBR GREEN I

Thaw the SYBR Green I solution. Spin the solution in a microcentrifuge. Dilute the stock to 1X in a pH 7.0-8.5 buffer. Store in a clear plastic polypropylene container. Prepare enough solution to just cover the top of the gel. After running electrophoresis, place the gel in the SYBR Green I solution at room temperature for 15-30 minutes depending on the thickness of the gel. View the gel using a 300 nm transilluminator or 254 nm epi-illuminator. There is no need for destaining.

SYBR® GOLD

EX/EM: 495/537

SYBR Gold is used after electrophoresis, not incorporated into the gel during electrophoresis. Although there is no data pertaining to the mutagenic properties of SYBR Gold, it should be treated as a mutagen and be handled with care.

STAINING WITH SYBR GOLD

Perform electrophoresis. Dilute stock SYBR Gold to 1X with TBE, TAE, or TE, keeping a pH of 7.0-8.0. Using a staining tray, submerge the gel completely in solution. Keep the gel and the solution out of the dark. Agitate the gel at room temperature. Stain for 10-40 minutes, depending on the thickness of the gel. View with a 300nm UV transilluminator or 254nm epi-illuminator.

GELSTAR™

EX/EM: 493/527 (DNA)
493/532 (RNA)

GelStar can be used after electrophoresis or be incorporated into the gel before electrophoresis. It is recommended to filter the gel buffer prior to casting the gel since GelStar is sensitive to particles in the gel.

PRE-STAINING WITH GELSTAR

Thaw the GelStar solution and spin it in a microcentrifuge. Prepare gel solution. Add GelStar to the cooling gel solution. For DNA, add stain solution so the final concentration is 1X. For RNA, the final concentration should be 2X. Cast the gel and run electrophoresis as practiced.

POST-STAINING WITH GELSTAR

Perform electrophoresis. Thaw and spin the GelStar stock solution. Prepare enough staining solution to completely submerge the gel. Dilute stock in TAE, TBE, or TE buffer. For DNA, the final concentration should be 1X, for RNA, 2X. Place the gel and stain in a staining tray. Agitate the gel while keeping the solution away from light. The staining process is about 30 minutes, longer if the gel is thick.

SILVER STAIN

Silver staining in nucleic acids is as sensitive as ethidium bromide. There are many silver staining kits available that yields higher sensitivity than traditional staining. Silver staining can only be used post electrophoresis.

POST-STAINING WITH SILVER STAIN

Reagents
1. Fixing solution: 500 ml methanol, 120 ml glacial acetic acid, 50 g glycerol. Add distilled water to bring the volume to 1 l.
2. Staining solution:
a. Mix 50 g sodium carbonate and distilled water to bring the volume to 1 l.
b. Mix 2.0 g ammonium nitrate, 2.0 g silver nitrate, 10 g tunstosilic acid, 8 ml 37% formaldehyde. Add distilled water to bring the volume to 1 l.
c. Mix 1:1 of the above two solution. Prepare enough to completely submerge the gel.
3. Stop solution: Prepare 1% glacial acetic acid in distilled water.

Protocol [1]
Submerge gel in fixing solution for thirty minutes. Wash the gel with distilled water for twenty minutes. Using a staining tray, completely submerge gel in the staining solution until the bands appear. Place the gel in the stop solution for five minutes. Destain the gel in distilled water and dry.

PROTEIN DETECTION IN GELS

COOMASSIE BRILLIANT BLUE

Coomassie blue non-specifically binds to proteins. It is faster and easier than silver staining. It is not as sensitive a silver staining. Rapid Coomassie blue staining can detect protein bands after 5-10 minutes after the initial staining. The sensitivity is slightly reduced with rapid staining.

STANDARD COOMASSIE BLUE STAINING

Reagents
1. Fixing solution: 50% (v/v) methanol, 10% (v/v) acetic acid, 40% distilled water.
2. Staining solution: 0.05% (v/v) Coomassie brilliant blue R-250, 50% (v/v) methanol, 10% (v/v) acetic acid, 40% distilled water. Dissolve Coomassie brilliant blue in methanol. Add acetic acid and water. Store up to six months.
3. Destaining solution: 7% acetic acid, 5% methanol, 88% distilled water.

Protocol [2]
Perform polyacrylamide electrophoresis. In a plastic container, place gel and enough fixing solution so the gel floats freely. Agitate for 2 hours on a rotary shaker. Pour out fixing solution, add staining solution to the container. Resume agitation for 4 hours. Pour out staining solution. Rinse gel with 50 ml of fixing solution. Pour out fixing solution and add destaining solution. Cover the gel in destaining solution and agitate for 2 hours. Pour out destaining solution, add fresh destaining solution. Continue the destaining process until clear blue bands and background appear.

RAPID COOMASSIE BLUE STAINING

Reagents
1. Fixing solution: 25% (v/v) isopropanol, 10% (v/v) acetic acid.
2. Rapid staining solution: 10% (v/v) acetic acid, 0.0006 (w/v) Coomassie blue G-250.

Protocol
Perform polyacrylamide electrophoresis. In a container, submerge the gel in the fixing solution. Shake gently at room temperature, 10-15 minutes for a 0.75-1.0 mm thick gel, 30-60 minutes for a 1.5 mm thick gel. Pour out fixing solution, add rapid staining solution. Shake gently for 2 hours to over night, until the desired visibility of the bands is obtained. Pour out staining solution. Cover the gel with 10% acetic acid to destain the gel. Shake gently for 2 hours. Repeat destaining process until a clear background appears.

SILVER STAIN

Silver staining is the binding of silver ions to the sulfhydryl and carboxyl groups of the proteins. It is more sensitive than Coomassie blue, detection limit at 2-5 ng/protein band. Silver staining is also more detrimental than Coomassie blue. Gloves and fume hood should be used while performing the staining process.

Reagents
1. Fixing solution: 50%(v/v) methanol, 10% (v/v) acetic acid, 40% water
2. Destaining solution: 7% acetic acid, 16.5% methanol, 78.5% water
3. Silver nitrate solution: add 3.5 ml concentrated NH4OH to 42 ml of 0.36% NaOH and bring to 200ml with water. Mix, slowly add 8 ml of 19.4% (16g/8ml) silver nitrate.
4. Developing solution: 0.5g sodium citrate, 0.5ml 37% formaldehyde solution, water to 100ml
5. 10% glutaraldehyde
6. Kodak Rapid Fix Solution A

Protocol [3]
Run polyacrylamide electrophoresis. Place the gel and fixing solution in a staining container. There should be enough solution to submerge the gel. Agitate on an orbital shaker for 30 min. Pour out fixing solution and pour in destaining solution. Agitate on an orbital shaker for 60 min. Pour out destaining solution and pour in 10% glutaraldehyde and agitate under a fume hood for 30 min. Pour out glutaraldehyde solution and wash 4 times with water, 30 min per time. Pour out water, add enough silver nitrate solution to submerge the gel. Vigorously shake the gel for 15 min. Wash the gel 5 times with deionized water, 1 min per time. Dilute 25 ml of developing solution with 500 ml water. Shake vigorously until the desired intensity of the band appears. Change the solution if it turns brown. Transfer the gel to Kodak Rapid Fix Solution A for 5 min. Photograph gel.

SYPRO® ORANGE

EX/EM: 470/560

SYPRO Orange is a fluorescent stain for protein analysis. It is much more sensitive than the traditional silver and coomassie blue stains. SYPRO Orange is also less time consuming, 10-60 minutes as opposed to overnight staining required by certain gels.

STAINING WITH SYPRO ORANGE

Dilute stock SYPRO Orange solution o 1:5000 in 7.5% acetic acid. Pour the staining solution in a small staining tray. Place the gel in the staining tray and cover with aluminum foil. Gently agitate the gel at room temperature for

10-60 minutes. The length of time is dependent on the thickness of the gel. Rinse briefly with 7.5% acetic acid and view with a 300nm transilluminator.

SYBR Green, SYBR Gold, and SYPRO Orange are registered trademarks of Molecular Probes. For more information please visit their website at http://www.probes.com
GelStar is a trademark of FMC BioProducts. For more information please visit their website at http://www.bioproducts.com

REFERENCES

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2. Ausubel, Frederick M. ed. Short Protocols in Molecular Biology. "Coomassie Blue Staining" John Wiley & Sons: New York, 1992.
3. Short Protocols in Molecular Biology. "Silver Staining" John Wiley & Sons: New York, 1992.