

GelBond® PAG Film

Introduction

GelBond® PAG Film is a patented, transparent, flexible polyester film designed to support polyacrylamide, Long Ranger™, ProSieve™ 50, or MDE™ Gels¹. The acrylamide monomers covalently attach to the coating on the film during the polymerization process so that slab gels of commonly used acrylamide concentrations can be easily handled without tearing. Gels remain permanently attached to the film through electrophoresis and all subsequent fixing, staining, destaining and drying procedures. Gels retain their original dimensions (except thickness) after drying. Dried gels are suitable for densitometric scanning, autoradiography, projection or permanent mounting in laboratory notebooks.

GelBond® PAG Film can be used in denaturing and nondenaturing electrophoresis and isoelectric focusing techniques. However, the 0.2 mm thick polyester film is neither electrically conductive nor porous, so it cannot be used in electrophoretic destainers or in electrophoretic or capillary blotting techniques. Gels can be stripped off GelBond® PAG Film by using a thin wire or cheese cutter².

GelBond® PAG Film will fluoresce when exposed to UV light. This background fluorescence may interfere with visual detection of fluorescent proteins or nucleic acids stained with ethidium bromide. If photography is used, background fluorescence can be screened out by using red, orange (e.g. Wratten® Gelatin Filter), and UV camera filters. GelBond® PAG Film will not transmit light of less than 310 nm, so nucleic acid gels stained with ethidium bromide should be inverted (gel side down) onto the light box in order to expose the separated sample bands to full light intensity. The fluorescing nucleic acid bands can be photographed through GelBond® PAG Film using this method.

GelBond® PAG Film can be used with bisacrylamide (N, N'-methylene-bis-acrylamide). With bisacrylamide, the use of a vacuum slab dryer is necessary in order to dry gels above 7%-8% T without cracking.

Product Stability

GelBond® PAG Film is sensitive to heat and light. It is packaged in a light-barrier wrapper, and if stored in a drawer at room temperature (<26°C), will remain stable for at least two years from date of manufacture. Although packages are marked "light sensitive", exposure to room lighting for a few hours does not deactivate the coating. Once a gel is cast on GelBond® PAG Film, the film is no longer sensitive to light.

Mounting GelBond® PAG Film in Casting Apparatus

Gels must be cast on the treated, hydrophilic side. GelBond® PAG Film is packed with the treated side up with paper interleaving to protect the treated surface. If uncertain, the hydrophilic side can be confirmed by applying a drop of water and noting that it spreads on the hydrophilic side, but beads up on the hydrophobic side. Always handle GelBond® PAG Film by the edges or with gloves to avoid getting fingerprints on the treated surface.

Studier-Design Chambers

Studier-design³ electrophoresis chambers use a notched glass plate to connect the upper buffer reservoir with the gel. In these chambers, GelBond® PAG Film should be mounted on the unnotched glass plate. Substitute for chambers a shorter plate for a notched plate. Mount GelBond® PAG Film onto the larger-outer plate. The film should be the same size as the unnotched glass plate or just slightly smaller.

1. Place a few drops of distilled water, 0.1% Triton® X-100 nonionic detergent, or 5% glycerol solution on the unnotched glass plate.
2. Without getting fingerprints on the GelBond® PAG Film, lay a sheet squarely on the glass plate, with the **hydrophilic** side facing up.
3. With the paper interleaving over the GelBond® PAG Film, wipe or roll (with a rubber roller) **firmly** from the center to the edges to squeeze out excess fluid and air bubbles. Wipe up any fluid squeezed out at

the edges. Remove the paper interleaving and clean off any fluid from the GelBond® PAG Film surface. **The film must be tightly adhered to the glass plate.**

4. Lay spacers on the GelBond® PAG Film and assemble the casting cassette as usual. From this point on, gel casting is the same as without GelBond® PAG Film.

Hofer-Design Chambers

Owners of Hofer SE design⁴ series vertical electrophoresis chambers with cam-gasket seals, such as those sold by Hofer, should use the following procedure to assure tight adhesion of GelBond® PAG Film in the cassette. This Scotch® Tape procedure, developed by Rosenblum⁵, has allowed his lab to run hundreds of gels without leakage.

1. GelBond® PAG Film should be cut slightly smaller than the dimensions listed for the glass plates. This is important to prevent the film from overlapping the glass plates, which can lead to buckling by the side clamps or the rubber gaskets at the top and bottom of the apparatus.
2. Use the following sequence to place GelBond® PAG Film on the glass plate.
 - a. Cut a strip of double-sided Scotch® Tape approximately two inches longer than the width of the glass plate.
 - b. Holding the tape taut, align it with the bottom edge of the glass and lay it in place. This tape will attach the GelBond® PAG Film to the glass and prevent acrylamide solution from channeling behind the film during casting.
 - c. Put a few drops of water in the center of the glass plate.
 - d. Position the GelBond® PAG Film (hydrophilic side facing up) so that the bottom edge is aligned with the bottom edge of the glass. Using a small roll of cotton gauze or soft paper tissues, press down firmly to seal the film to the tape. Press out the excess fluid so that a **very tight seal** is formed between the glass and the film. Make sure that no air bubbles or pockets of water remain except a small line of air along the edge of the Scotch tape. Wipe any water off the GelBond® PAG Film.
 - e. Grease the side spacers as follows: Dip the top ends of each spacer into Cello-Seal™ Grease (or similar product) to provide a small dab of grease on the back side. Apply a thin line of grease down the back side of the spacer (conveniently applied by syringe or by streaking it on with the rounded end of a test tube).

- f. Lay the spacers in place with the greased side on the GelBond® PAG Film, pressing down at the top to squeeze the dabs of grease from **step e**. Wipe off excess grease at the top. Apply a thin line of grease down the top side of each spacer.

- g. Trim off the excess tape, lay the second glass plate down properly, and assemble the mold according to the chamber's instructions. Tighten all clamp screws only tight enough to get a good seal with the greased spacers. Over tightening can cause plates to crack.

- h. Before camming the mold into the casting stand (or upper buffer tank, later), place dabs of grease on the corners of the glass plate and spacer ends to prevent leakage. (A light coating of grease on the rubber gasket is also helpful.)

3. Insert the comb at a slight angle away from the GelBond® PAG Film surface. This will align the teeth and prevent catching them on the edge of the film. **Insert the comb prior to filling the casting assembly** to prevent solution from leaking behind the GelBond® PAG Film.

Processing Bisacrylamide Gels Vacuum Drying of High-Concentration Bisacrylamide Cross-Linked Gels.

To dry bisacrylamide cross-linked gels attached to GelBond® PAG Film on a vacuum slab dryer, use the following procedure.

- a. Cover the glycerol-soaked gel with a prewet sheet of porous cellophane (available from vacuum slab dryer dealers), dialysis membrane, or chromatography paper. Plastic food wrap will **not** work.
- b. Place several sheets of chromatography paper on the gel dryer and position the gel over these so that the GelBond® PAG Film is away from the vacuum source.
- c. Dry the gel under vacuum according to the manufacturer's instructions. GelBond® PAG Film can withstand dryer temperatures of 80°C. Drying times generally range from about one hour for a 0.75 mm thick gel to about five hours for a 3.0 mm thick gel. Whereas porous cellophane will adhere to the dried gel permanently, the chromatography paper can be removed by soaking the dried gel briefly in distilled water and gently peeling the paper off.

Staining and Destaining Procedures Coomassie® Brilliant Blue Stain

Destain solution is changed 3-4 times to expedite the process. Gels can be left in destain overnight without causing the gel to disadhere from the GelBond® PAG Film. All gels must be soaked in glycerol-preserving solution for ≥1.5 hours before drying.

Silver Stain (Adapted from J.H. Morrissey).⁶

Washing and staining times need to be adjusted for differing gel thickness or concentrations, since diffusion can only occur from the exposed gel surface. In general, it is best to cast a thin gel (≤ 1 mm) so that developer and stop solutions can readily diffuse into the gel. This results in better sensitivity and lower background staining.

Compatibility

Periodic Acid Silver Stain

GelBond[®] PAG Film can be used with the Tsai and Frasch⁷ periodic acid silver stain procedure for lipopolysaccharide. It is very important to increase washing times in this application to prevent high background stain deposition in the GelBond[®] PAG Film-supported gels.⁸

Kodavue[™] Nickle Stain

GelBond[®] PAG Film is chemically incompatible with the Kodak[®] Kodavue Nickle Stain Kit.⁹

Sucrose

Gradient gels containing 15% sucrose as a weighting solution of the high-concentration end have been reported to disadhere from GelBond[®] PAG Film. The gels cited contained 5%-20% polyacrylamide, cross-linked with bisacrylamide. For best results when casting gels on GelBond[®] PAG Film, 15% glycerol should be used instead of sucrose.

Urea

Urea can be used at high concentrations of 8 M with GelBond[®] PAG Film and polyacrylamide gels without seriously interfering with adhesion. However, urea is commonly employed in conjunction with other ingredients that do reduce adhesion (i.e. nonionic detergents and alkaline ampholytes).

Nonionic Detergents

Triton[®] X-100 and Nonidet[™] P-40 (NP-40) Detergents are popular in IEF of sparingly soluble proteins. They diminish gel adhesion to GelBond[®] PAG Film, apparently by blocking the reactive surface. (Gel polymerization proceeds as usual.) Nonionic detergents with urea are said to have a similar effect on silanized polyester and other plastic supports.

Poor adhesion at a 2% NP-40 level can be overcome by reducing the NP-40 level to 0.5% or lower. Reliable binding of polyacrylamide gels to GelBond[®] PAG Film has been obtained in the presence of 8 M urea with 0.5% NP-40 or the zwitterionic detergent CHAPS,¹⁰ by increasing the catalyst concentration to 2 to 3-fold. Both CHAPS and CHAPSO¹¹ are compatible with GelBond[®] PAG Film.

Another approach is to cast the gel without detergent, then equilibrate it in a bath of detergent, urea and alkaline ampholytes for a few hours.

Alkaline Ampholytes

Alkaline ampholytes interact with polymerizing acrylamide solutions to inhibit gel adhesion to GelBond[®] PAG Film. This is believed to be due to amino groups in the ampholytes competing with acrylamide to bind to the reactive GelBond[®] PAG Film surface. The interference can occur with ampholytes above pH 8 in narrow or wide-range pH gradients.

Good adhesion can be obtained by using **no more than 2.5% w/v ampholytes** in the gel, while including higher than normal levels of ammonium persulfate (0.7-1.0 mg/ml of gel-forming solution) and TEMED (1 μ l/ml). Since excess salts can disrupt IEF results, the gel can be soaked in a solution of ampholytes or prefocused for 15 minutes at 500 V to remove salts before samples are applied.

Immobiline[®] Gels

Immobilized pH gradient isoelectric focusing (IPG-IEF) is a specialized technique for greatly expanding the scale of narrow-range IEF. The technique employs Immobiline Ampholytes (Amersham-Pharmacia Biotech) and GelBond[®] PAG Film. Immobilized pH gradient gels have been frequently reported for nondenaturing IEF. Modifications of the technique have been reported under conditions that solubilize and denature most proteins. By reducing TEMED and APS, conditions have been determined which allow adequate time to pour even very viscous gel solutions containing 4% acrylamide, 8 M urea, and 2% NP-40. Under these conditions the gel adheres firmly to the GelBond[®] PAG Film backing.¹²

Trichloroacetic Acid (TCA)

TCA is frequently used as a fixative after IEF or peptide electrophoresis. High TCA concentrations can damage gel adhesion to GelBond[®] PAG Film. Fixatives with 10% TCA or less can normally be used without problems if fixing time is no longer than 30 minutes at room temperature. IEF gels containing other ingredients that weaken adhesion (such as nonionic detergents) may be digested off the support even by 10% TCA.

For peptides, TCA is typically employed at levels as high as 25% which can cause disadhesion in as little as 30 minutes. Gels left in more dilute fixatives (5%) for several hours to wash out ampholytes may also disadhere. **Refrigeration of the fixative bath and minimizing the TCA concentration and exposure time can prevent disadhesion.**

TCA is sometimes included in the solvent for Coomassie[®] Brilliant Blue G-250 stain. We do not recommend use of this procedure.

Agarose-Acrylamide Composite Gels

Neither GelBond® PAG Film nor standard GelBond® Film is designed for, or reliable with, composite gels. For composites in which acrylamide is allowed to polymerize above the gelling temperature of the agarose, GelBond® PAG Film may adhere the gel better than standard GelBond® Film.

Conversely, if the agarose is allowed to gel before the acrylamide polymerizes, standard GelBond® Film is preferable. However, there is less adhesion of composite gels than agarose gels.¹³

Dehydrating Solutions

Severe shrinkage followed by expansion is often the cause of disadhesion from GelBond® PAG Film. The following solutions are all known to shrink gels so that they tear off the support when reswollen.

1. Alcohol (methanol, ethanol, isopropanol) solutions of 45% or greater concentration.
2. DMSO (dimethylsulfoxide).
3. Perkin Elmer' En³HANCE® autoradiography enhancer. (Perkin Elmer also reports that fluorography with EN³HANCE is seriously inhibited by GelBond® PAG Film. The interference is more pronounced with tritium.¹⁴ However, many customers have reported satisfactory results with ³⁵S.)

Gel shrinkage by solution dehydration is analogous to final gel drying. Inclusion of 5% glycerol preceding the dehydrating step has prevented disadhesion in many instances. Dehydration by DMSO followed by rehydration to precipitate PPO is too severe, however, for the glycerol approach. No adequate preventive measure is known for DMSO-caused disadhesion.

High Degrees of Bisacrylamide Cross-Linking

Highly cross-linked 10% gels (19:1 acrylamide to bisacrylamide) have been reported to shrink severely overnight in 25% isopropanol/10% acetic acid. If bonded to GelBond® PAG Film, these gels curl up and crack when dried, even after being soaked in as high as 20% glycerol and carefully dried on a vacuum slab dryer.¹⁵

References

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Related Products

Long Ranger™ Solution
ProSieve™ 50 Solution
MDE™ Gel Solution

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