GelBond® PAG Film

Introduction

GelBond PAG film is a patented, transparent, flexible polyester film designed to support polyacrylamide, Long Ranger®, ProSieve® 50, or MDE™ gels. 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.
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), however, with bisacrylamide it is necessary to use a vacuum slab dryer 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 three years. Although packages are marked “light sensitive”, exposure to room lighting for a few hours does not deactivate the coating. (Every lot is tested to ensure activity is maintained after 100 hours of continuous fluorescent lighting.) Once a gel is cast on GelBond PAG film, the film is no longer sensitive to light.

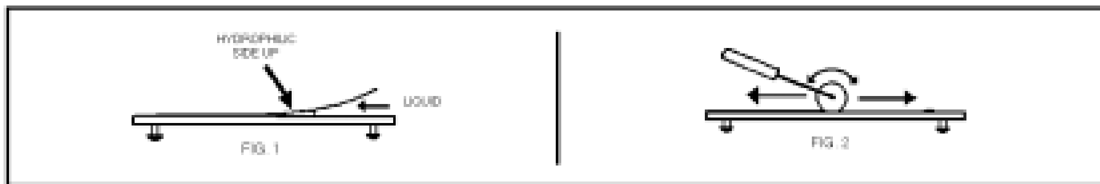
Mounting GelBond PAG Film in Casting Apparatus.

Note: 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-design[3] 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. A modified version of the Studier chamber, sold by BRL and others, substitutes a shorter plate for the notched one. When using these chambers, the GelBond PAG film should be mounted 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.
2. Without getting fingerprints on the GelBond PAG film, lay a sheet squarely on the glass plate, with the hydrophilic side facing up (Fig. 1).
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 (Fig. 2). Wipe up any fluid squeezed out at the edges. Remove the paper interleaving and clean off any fluid from the GelBond PAG 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.



Hoefer-Design Chambers

Owners of vertical electrophoresis chambers with cam-gasket seals, such as the SE series from Hoefer[4], should use the following procedure to assure tight adhesion of GelBond PAG film in the cassette. This Scotch® tape procedure, developed by Rosenblum[5] 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.

Instructions for Preparing SDS-Polyacrylamide Gels.

1. Preparation of Acrylamide/Bisacrylamide Gels.
   a. Prepare your usual stock solution of acrylamide and bisacrylamide.

Caution: Acrylamide is a neurotoxin and must be handled with care, using protective rubber gloves and adequate ventilation.

2. Laemmli SDS-PAGE Gels
   a. Cast the concentration resolving gel you require.
   b. Carefully overlay the gel-forming solution with distilled water, separating gel buffer or water-saturated 2-butanol.
   c. Allow the gel to polymerize for at least one hour undisturbed. Note: If the gel will not

be run until a later day, seal the top of the mold with plastic wrap at this step. (Do not cast the stacking gel until you are ready to run the gel, because diffusion of buffers will ruin the gel.)

d.Cast the stacking gel.

3. Sample Preparation
   a. Heat samples in a boiling water bath for 3-5 minutes in pH 6.8 sample buffer (0.0625 M Tris-HCI, 2% SDS, 10% glycerol, 2.0% 2-mercaptoethanol, 0.002% bromophenol blue and 0.001% phenol red tracking dyes).
   b. Apply samples (typically 10 µl) to the sample wells. For Coomassie® Brilliant Blue staining, total protein sample concentrations around 250 µg/ml are typical. For silver stain, load 50 µg/ml.
4. Electrophoresis
   a. Fill the buffer tanks with electrode buffer (0.025 M Tris-HCI, 0.192 M glycine, 0.1% SDS, pH 8.3; do not adjust the pH).
   b. Electrophoresis at 20-25 mA for approximately 1 3/4 hours or until the phenol red line nearly reaches the end of the separating gel.
5. Gel Processing (For Low < 8%T) Concentration Bisacrylamide Gels)
   a. Fix, stain, and destain the gel, then soak it in 2.5-5% glycerol solution for about two hours. (Glycerol may be included in the destain solution, thus eliminating the extra step.) Briefly rinse the gel with distilled water to remove surface glycerol.
   b. Clamp the gel to a glass plate, gel side out, and dry in a 60° C oven (preferably forced-air draft) for about two hours, or air dry on a bench top for about 24 hours.
6. 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 pre-wet 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

For SDS-PAGE Gels:

Fix/Stain Solution	Volume	Conc.
Coomassie BB R-250	5 g	0.5%
Methyl Alcohol (99%)	400 ml	40%
Glacial Acetic Acid	100 ml	10%
Distilled water	500 ml	

Mix and filter solution through Whatman® #4 paper

Destain	Volume	Conc.
Methyl alcohol	200 ml	20%
Glacial acetic acid	50 ml	5%
Distilled water	750 ml	

Preserving Solution	Volume	Conc.
Glycerol	25 ml	2.5%
Distilled water	975 ml	

Gels are allowed to stain with mild agitation for 1-2 hours, followed by destaining for 4 hours to overnight. Destain solution is changed 3-4 times to expedite the process. Gels can be left in destain overnight without causing the gel to detach from the GelBond PAG film. All gels must be soaked in glycerol-preserving solution for > 1.5 hours before drying.

PAGE-IEF Gels (130 x 110 x 0.5 mm)

Fixative Solution	Volume	Conc.
TCA (Trichloroacetic acid)	115.0 g	11.50%
Sulfosalicylic acid	34.6 g	3.46%
Distilled water	to 1,000 ml	
Processing Gel	Volume	Time
	100 ml/gel	15min

Note: TCA causes the gel to detach from GelBond PAG film. This can be avoided by limiting exposure times to less than 30 minutes and refrigerating during fixing.

Rinse

Distilled Water	1.5 LDH_2O/gel	2 x 30 min.

Stain

Coomassie BB R-250	0.5 g	0.05%
Isopropyl alcohol (99%)	250 ml	25%
Glacial acetic acid	100 ml	10%
Distilled Water	650 ml	

Mix overnight; filter through Whatman #4 paper.

Processing Gel	Volume	Time
	100ml/gel	60 min.

Destain

Methanol	400 ml	40%
Glacial acetic acid	100 ml	10%
Distilled water	500 ml	

Mix.

Processing Gel	Volume	Time
	300 ml	120+ min

Preserving Solution

Glycerol	25 ml	2.5%
Distilled water	975 ml	

Mix.

Processing Gel	Volume	Time
	500 ml	60+ min

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

Gels can be fixed and stained in the same acid-cleaned glass (or plastic) container under normal room lighting conditions. Gloves should be worn when handling the gels to avoid stained fingerprints. All solutions should be filtered to remove particulates.

The volumes of stop solution and developer must be balanced to bring the pH to neutrality. The reaction will not stop at alkaline pH, and bleaching of the stain can occur at acidic pH.

The processing times given are for 145 x 165 x 1 mm gels (16% T) cast on GelBond PAG film. 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.

Step / Formula	Volume	Conc.
First Fixative		
Methanol	500 ml	50%
Glacial acetic acid	100 ml	10%
Distilled water	400 ml	
	Volume	Time
Processing Gel	100 ml/gel	30 minutes
Second Fixative		
Methanol	50 ml	5%
Glacial acetic acid	70 ml	7%
Distilled water	880 ml	
	Volume	Time
Processing Gel	100 ml/gel	30 min or overnight
Rinse		
Processing Gel	1.5 L/gel DH_2O	2+ hours, or overnight
Prestain		
Dithiothreitol	1.0 mg	0.5%
Distilled water	200 ml	
Processing Gel	Volume	Time
	100 ml/gel	30 minutes
Stain		
Silver nitrate	0.2 gm	0.1% w/v
Distilled water	200 ml	
Processing Gel	Volume	Time
	100 ml/gel	30 minutes
Rinse		
1. Distilled water	100 ml/gel	4 x 15 sec.
2. Developer	100 ml/gel	2 x 15 sec.
Developer		
Sodium carbonate	30 g	3%
Distilled water	995 ml	
Formaldehyde (37%)	0.5 ml	0.0185%
Processing Gel	Volume	Time
	100 ml/gel	Until stained
Stop Solution (added to developer solution)		
Citric acid	48.3 g	2.3M
Distilled water	100 ml	
Processing Gel	Volume	Time
	5 ml/100 ml	10 minutes
Rinse		
Distilled water	500 ml/gel DH_2O	3 x 10 min
Preserving Solution		
Glycerol	25 ml	2.5%-Distilled
water	975 ml	
Processing Gel	Volume	Time
	500 ml/gel	1.5 + hour

(If wet gel will be stored, add 0.3 g Na_2CO_3 to prevent bleaching.)

Compatibility

Periodic Acid Silver Stain

GelBond PAG film can be used with the Tsai and Frasch[7] 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-supported gels.[8]

Kodavue® Nickel Stain

GelBond PAG film is chemically incompatible with the Kodak® Kodavue nickel stain kit.[9]

Sucrose

Gradient gels containing 15% sucrose as a weighting solution of the high-concentration end have been reported to detach 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 CHAPSO[11] 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 µl/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 detachment in as little as 30 minutes. Gels left in more dilute fixatives (5%) for several hours to wash out ampholytes may also detach. Refrigeration of the fixative bath and minimizing the TCA concentration and exposure time can prevent detachment.

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 detachment from GelBond PAG film. The following solutions are all known to shrink gels so that they tear off the support when re-swollen.

- Alcohol (methanol, ethanol, isopropanol) solutions of 45% or greater concentration.
- DMSO (dimethylsulfoxide).
- NEN Research Products En^{3}HANCE® autoradiography enhancer. (NEN also reports that fluorography with EN^{3}HANCE is seriously inhibited by GelBond PAG film. The interference is more pronounced with tritium.[14] However, many customers have reported satisfactory results with ^{35}S.)

Gel shrinkage by solution dehydration is analogous to final gel drying. Inclusion of 5% glycerol preceding the dehydrating step has prevented detachment 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 detachment.

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 at 20% glycerol and carefully dried on a vacuum slab dryer.[15]

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BioWhittaker Molecular Applications
191 Thomaston Street
Rockland, ME 04841
Technical Service

Ph: 800-341-1574
Fax: 800-362-5552
Outside the USA: 207-594-3400
Ph: 800-521-0390
e-mail: biotechserv@biowhittaker.com

BioWhittaker Molecular Applications ApS
Risingevej 1
DK-2665 Vallensbaek Strand
Denmark

Ph: +45 43 56 74 00
Fax: +45 43 56 74 03

Web: www.cambrex.com

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