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GelBond® Film

Casting Agarose Gels on GelBond Film

I. Introduction

GelBond® Film is a transparent, flexible plastic film designed to support agarose gels. This convenient, ready to use film has a specially treated hydrophilic side that firmly adheres agarose gels as they form. Gels remain adhered to the film from the time they are cast through all electrophoresis or immuno-diffusion, fixing, staining, and destaining procedures.

GelBond® Film provides many advantages over the use of glass plates alone. Gels cast on it are easy to handle and can be dried and stored permanently. GelBond® Film often eliminates the need for photography. The use of GelBond® Film is particularly suitable for gels stained with Coomassie® Brilliant Blue or agarose silver stain. Agarose gels can be silver stained only after drying onto GelBond® Film.

GelBond® Film is non-porous and can not be used with electroblotting or capillary blotting applications.

GelBond® Film is intended for use with agar and agarose gels. GelBond® Film is recommended for most agarose gel applications including gel diffusion, electroimmunodiffusion, serum protein electrophoresis, isoelectric focusing and nucleic acid electrophoresis.

GelBond® PAG Film is available for use with polyacrylamide gels.

II. Vertical Cassette Casting

The casting cassette consists of two glass plates, GelBond® Film, spacers, and clamps.

Assemble the cassette as follows:

1. Spread a few drops of distilled water or 0.1% nonionic detergent solution on the back plate.
2. Lay a sheet of GelBond®
3. Film, cut slightly smaller (1 mm-2 mm) than the glass plate, onto the plate with the hydrophilic side up (water droplets spread on the hydrophilic side but bead up on the hydrophobic side).
4. Cover the GelBond® Film with a sheet of blotting paper or the interleaving paper supplied with the GelBond® Film and firmly roll with a rubber roller or wipe with tissues to squeeze out any air bubbles and excess fluid from behind the GelBond® Film. Carefully wipe off any excess liquid at the edges. The GelBond® Film should now be tightly adhered to the glass plate.
5. Arrange the side spacers and the bottom spacers on top of the GelBond® Film.
6. Place the top plate over the bottom plate.
7. Tape the sides of the cassette with separate pieces of tape.
8. Tape the bottom of the cassette with a separate piece of tape. This allows the tape on the bottom to be removed for electrophoresis without disturbing the tape at the sides of the gel.
9. Clamp the plates together.

Casting a vertical agarose gel

1. Prepare agarose solution according to the manufacturer's instructions and cool to 60ºC.
2. Pre-warm the assembled cassette and a 60 ml syringe for 15 minutes by placing in a 55ºC oven or by using a heat gun.
3. Pour agarose into a pre-warmed 60 ml syringe fitted with a 16-gauge needle.
4. Wedge the needle tip between the plates in the upper corner of the cassette with the needle opening directed toward the back plate.
5. Angle cassette and inject the agarose solution at a moderate, steady rate allowing the agarose to flow down one side spacer.
6. Fill until the agarose solution goes just above the glass plates.
7. Insert one end of the comb, then slowly insert the rest of the comb until the teeth are at an even depth.
10. Insert the comb into the agarose to the minimal depth necessary to accommodate your samples.
11. Place extra clamps on the side of the glass plates to hold the comb in place.
12. Cool the gel at room temperature for 15 minutes.
13. Place the gel at 4ºC for 20 minutes.

14. Remove the clamps at the top of the gel.
15. Remove excess agarose with a scalpel or razor blade.
16. Squirt running buffer in the spaces between the comb and the gel.
17. Slowly and gently lift the comb straight up. Allow air or buffer to enter the well area to release the vacuum which forms between the agarose and the comb.
18. Flush wells with running buffer.
19. Store gels overnight in a humidity chamber or in a sealed bag with a buffer-dampened paper towel.

Preparing for electrophoresis

NOTE: Since agarose does not adhere well to glass, leave as many clips in place as possible. For some electrophoresis chambers, it is helpful to seal the spacers at the top of the gel with molten agarose.

1. Remove the tape at the bottom of the cassette.
2. Place the cassette into the chamber at an angle to minimize the number of bubbles which can collect in the well area.
3. Rinse out well area with a running buffer.

Disassemble the casting cassette as follows

1. Remove the tape and all clamps.
2. With the cassette lying flat, insert a flat spatula between the glass plates and twist gently to break the seal. Carefully remove the top plate, leaving the gel and the GelBond® Film attached to the back plate.
3. Remove the spacers and lift the gel, attached to the GelBond® Film from the back plate.

III. Open Casting for Horizontal Isoelectric Focusing

As an alternative to using a casting cassette, gels can be formed by an open casting method. This technique is optimal for gels of 1.0 mm-1.5 mm thickness. However, there is a greater likelihood of nonuniformity and evaporative loss with this method. Gels must be cast on a level surface to assure a uniform thickness. Care must be taken to prevent the agarose solution from overflowing the edge of the GelBond® Film and running under the support film.

1. For open casting, adhere GelBond® Film to a glass plate as described previously.
2. As with cassette casting prewarm the plate with GelBond® Film attached prior to gel casting.
3. After casting and once the gel has set, refrigerate the gel in a humidity chamber for one hour prior to use.

IV. Preparation for Horizontal Electrophoresis

1. Trim the gel with a razor blade to ensure straight edges.
2. Spread a small volume of 0.1% nonionic detergent or distilled water on the cooling platen or bed of the electrophoresis chamber.
3. Lower the gel on the wetted area. Avoid trapping air under the GelBond® Film. Wipe excess fluid from the edges of the film.
4. Blot the surface of the gel briefly with a sheet of fine-grained blotting paper.
5. Apply samples by using either a sample application mask or filter paper sample applicator pieces.
6. Run the electrophoretic separation.

V. Fixing and Staining

General instructions for fixing and staining

1. Soak the gel in the appropriate fixative for 2 minutes, then gently agitate for 10-20 minutes. **NOTE:** Prolonged exposure to TCA fixative can disadhere the gel from the GelBond® Film.
2. Wet a sheet of fine grained blotting paper with distilled water and place it on the gel surface. Overlay the blotting paper with 4-6 layers of absorbent paper toweling. Place a glass plate on top and weight it down with a 1 kg-2 kg weight for 20 minutes.
3. Remove the weight, glass plate and the paper toweling. Rewet the blotting paper thoroughly with distilled water and gently lift it off the gel surface.
4. Rinse the gel in a large volume of distilled water for 5-10 minutes with agitation to remove residual fixative (or ampholytes from IEF gels).
   To accelerate deproteinization of immunoelectrophoresis gels to assure a clear background, saline soakes and press blotting should be repeated 2-5 times.
5. Clamp the GelBond® Film, gel side out, onto a glass plate to prevent curling during drying. Dry the gel completely in a forced hot air oven (55ºC-60ºC) or on the lab bench overnight. Oven drying usually takes <30 minutes.
6. Transfer the dried gel to a stain bath for 10-30 minutes. Float the gel on the surface of the solution with the gel facing down into the stain. This prevents precipitated stain from settling on the gel.
7. Remove the stained gel, rinse it briefly in distilled water, destain for a few minutes as needed, then rinse briefly in distilled water.
8. Clamp the gel to a glass plate, gel side out and dry the gel in a forced-air oven for approximately 15 minutes or dry at room temperature overnight. Gels will crack if overdried. The gel can be read, stored or otherwise used without further treatment.

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VI. Special Techniques

A. Press Blot Transfer to Membranes

GelBond® Film is neither porous nor electrically conductive so it cannot be used with conventional or electrophoretic blotting procedures. However, unfixed proteins and nucleic acids can be transferred to nitrocellulose or other membranes by direct pressure blotting. This procedure is nearly identical to the press blot drying previously described, except that a sample binding membrane (e.g. nitrocellulose) is substituted for the sheet of blotting paper and no weight is placed on the glass plate. Proteins and nucleic acids are drawn out of the gel and onto the membrane, with approximately 20% of the proteins transferred after just 1½ minutes and up to 85% transferred after 35-40 minute blotting time.

B. Ultraviolet Light and Fluorescent Staining

- GelBond® Film exhibits background fluorescence when exposed to UV light. This background may interfere with visual detection of fluorescent bands such as cardiac isoenzymes 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® Film also blocks much of the light below 300 nm, so nucleic acid gels should be inverted (gel side down) onto the light box in order to expose the separated bands to full light intensity. The fluorescent bands photograph well through GelBond® Film using this method.
- To stain gels backed with GelBond® Film with GelStar®, SYBR® Green or SYPRO® Red Gel Stains, follow the directions for staining vertical gels in the stains instruction sheet.

VII. Troubleshooting

Adhesion

- For maximum adhesion cast agarose solutions at 60ºC-65ºC.
- Always preheat casting cassette or glass plate with GelBond® Film attached to 60ºC-65ºC.
- Only cast gels on the hydrophilic side of GelBond® Film.
- Store GelBond® Film in a dry location.
- Prolonged exposure to TCA can disadhere gels from the film.
- Include 0.5%-2% glycerol in the final rinse or soak step to prevent gels from cracking during drying.
- Destain times may have to double to increase their efficiency.

GelBond® Film curl

- Avoid high temperature (>65ºC) drying and overdrying of the gel.
- Always clamp the gel to a glass plate while drying.
- To uncurl GelBond® Film cut from a roll, place the cut film, rewound in the opposite direction, into a beaker of boiling water for 5 minutes.
- Soak curled gels in 0.5%-2% glycerol and re-dry at room temperature.

Ordering Information

GelBond® Film, 0.2mm thick sheets 100 per package

Catalog No.	Size (mm)
53734	85 x 100
53745	110 x 125
53746	100 x 150
53748	110 x 205
53749	160 x 180
53759	125 x 245
53761	124 x 258

GelBond® Film, 0.2mm thick rolls, 16.5 meters long

Catalog No.	Roll Width
53740	102 mm
53750	127 mm
53760	152 mm
53780	203 mm

Related Products

GelBond® PAG Film
IsoGel® Agarose
Lonza Agarose

For Laboratory Use.

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