Published in final edited form as:

Methods Mol Biol. 2012 ; 869: 437–443. doi:10.1007/978-1-61779-821-4_37.

Long-term, buffer-less, wet gel storage in non-sealed polyethylene bags

Biji T. Kurien^{§,¶,&}, R. Hal Scofield^{§,¶,&}

[§]Department of Medicine, Diabetes and Endocrinology Section, University of Oklahoma Health Sciences Center;

[¶]Arthritis and Clinical Immunology, Oklahoma Medical Research Foundation;

[&]Department of Veterans Affairs Medical Center, Oklahoma City, Oklahoma, OK 73104, USA.

Summary

Electrophoresed gels are normally fixed, stained, destained and dried. Drying is normally carried out with commercial gel dryers or by drying between two cellophane sheets held together by two acrylic frames. Here, we report that stained and destained gels (7.5, 10, or 15%, denaturing or native gels; 0.4 or 1.5 mm in thickness) could be stored wet, unsealed, and without any storage buffer for several months at room temperature within flexible polyethylene bags without significant shrinking or protein diffusion. The gel remains hydrated because of the *de facto* sealing achieved by the polyethylene sheets (PS) adhering air-tightly to the gel on either sides. The microsaturation environment generated by the thin film of water molecules trapped between the gel and the PS, along with the nonporous nature of the PS, apparently protects the gel from cracking as well as shrinking significantly. The intensity of stained proteins increased during storage probably from the slight gel shrinkage observed. Wet gel storage is useful (a) when low abundance protein spots from multiple 2-dimensional electrophoresis gels have to be excised for in-gel tryptic digestion or electroelution and (b) for wet gel autoradiography. In addition to avoiding dryer contamination and saving drying time, these bags prevent the moist gel from sticking to X-ray film. Such storage could also prove useful for electrophoretic transfer of fixed and stained gels.

Keywords

SDS-PAGE; Coomassie Blue; Protein staining; Gel storage

1. Introduction

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (1) and 2-dimensional gel electrophoresis (2) have served as an invaluable analytical tool in basic and applied research. Gels are traditionally dried following electrophoresis, for purposes of scanning and/or documentation. A variety of drying methods have been used for this

[Reproduced from *Analytical Biochemistry*, 1998 (see **Reference 6**) with permission from Elsevier Limited, Oxford, OX5 1GB, UK].

Correspondence to: Biji T. Kurien, Oklahoma Medical Research Foundation, 825 NE 13th Street, MS 24, Oklahoma City, OK 73104, Phone: (405) 271-7394, Fax: (405) 271-7063, biji-kurien@omrf.org.

purpose, including the use of commercial gel dryers (3) and drying between two cellophane sheets held together by two acrylic frames. We observed that we could store stained and destained gels (0.4 or 1.5 mm in thickness, denaturing or native, 7.5, 10, or 15%) wet, unsealed, and without any storage buffer for several months at room temperature within flexible polyethylene bags without significant shrinking or protein diffusion.

Gels were obtained from various experiments to study the efficacy of polyethylene bags to serve as a long-term storage medium. First, isoelectric focusing (IEF) of human granulocyte membrane proteins was carried out in a tube gel in the first dimension followed by SDS-PAGE (10%, 1.5 mm thickness) in the second dimension. The gel was immunoblotted non-electrophoretically immediately following SDS-PAGE (4). After transfer, the gel was stained, destained, and stored in a polyethylene bag. Secondly, increasing concentrations of bovine serum albumin (BSA) was electrophoresed (7.5 and 10% SDS-PAGE gels, 1.5 mm thick), stained, destained, and stored in polyethylene bags. Thirdly, HeLa cell extract was electrophoresed in a 15% SDS-PAGE gel (1.5 mm thick), stained, destained, and stored. Finally, flat-bed native IEF (0.4-mm gel thickness) of rabbit anti-spectrin was carried out (5). After focusing was complete, the gel, still on the gel support film, was stained, destained, and stored.

Figure 1A shows a 2-DE gel after being placed in a polyethylene bag. The same gel, after storage for over 2 months in the polyethylene bag at room temperature is shown in Figure 1B. Similarly, Figures 2A and 2B show SDS-PAGE gel (10%) containing BSA before and after storage in a polyethylene bag for over a month, respectively. Similarly, storage of a 7.5% SDS-PAGE gel containing BSA and a 15% SDS-PAGE gel with HeLa cell extract analyzed on it in a polyethylene bag was also successful (data not shown). In addition, an electrophoresed native IEF gel (5) was also stored successfully in a polyethylene bag for over 2 months (data not shown). Several additional 10% SDS-PAGE gels were also stored similarly for at least 3 months and some more than 6 months. The gels did not cracking and display any significant change from its original form. The patterns of the protein bands did not change over this period, even though there was a slight shrinkage and increase in intensity of stained protein bands.

We also found that wet gel autoradiography with gels in polyethylene bags prevents the X-ray film from getting wet and thus prevents the film from sticking to the gel, unlike performing this with the radioactive gel wrapped in clear plastic wrap (unpublished observation). SDS-PAGE gels (precast) are sold stored in buffer inside sealed plastic bags or aluminum foil. Storage buffer is essential to provide a saturated atmosphere to prevent gel cracking (dehydration) or swelling (hydration). When gels are stored in polyethylene bags, the polyethylene sheets trap water molecules between themselves and the gels on either side and thus provide a microsaturation environment (6). The sheets adhere air-tightly to either sides of the gel to provide a *de facto* sealing. Furthermore, the sheets stick to themselves in the regions around the gel where liquid droplets from the gel drip into the bag (when the gel is placed in the bag). The polyethylene sheets do not appear to be porous and therefore are able to maintain this microsaturation atmosphere for several months. We observed an increase in protein stain intensity in the gels stored in the polyethylene bags. This is more than likely due to the slight shrinkage observed (6).

Even though all these gels were stored at room temperature, we observed that they could be stored at 4°C without significant shrinkage for over a month. However, there was significant shrinkage (without cracking) when stored at –20°C for over a month (data not shown).

Wet gel autoradiography with these bags can help avoid possible contamination of gel dryers, reduce exposure to radioisotopes and save time/effort (by not having to dry the gel). Storing gels in this manner also enables electrophoretic transfer of fixed and stained gels at a future date, if necessary (7). Dried gels, of course, can be rehydrated and used. However, we observed that filter paper (used for drying) remnants remain stuck to the gel after rehydration, which necessitates painstaking effort to remove them to prevent the gel from tearing.

Storage of gels in polyethylene bags permits the investigator to compare gels for reproducibility of protein spots and also to excise protein spots when enough number of gels has been collected (especially for protein of low abundance). Various kinds of gels, thin and thick gels, gels of different concentrations, and also denaturing and native gels can be stored in polyethylene bags.

2. Materials

Prepare all solutions using ultrapure water (prepared by purifying deionized water, to attain a sensitivity of 18 M Ω cm at 25°C) and analytical grade reagents. Prepare and store all reagents at room temperature (unless indicated otherwise). Diligently follow all waste disposal regulations when disposing waste materials. We do not add sodium azide to reagents.

1. SDS-PAGE gels (7, 10 and 15%; 1.5 mm thick) (1).
2. Isoelectric focusing tube gels (2).
3. Flat bed, native isoelectric focusing gel (5).
4. Flexible polyethylene bag (Fisher Scientific Company, Springfield, NJ).
5. Granulocyte membranes (8).
6. HeLa cell extract.
7. Coomassie Brilliant Blue (CBB) stain: 0.05% (CBB) in 25% methanol/10% acetic acid (*see* Note 1).
8. Destaining solution: 25% methanol/10% acetic acid.

3. Methods

All procedures are carried out at room temperature unless otherwise specified.

3.1. Two dimensional gel electrophoresis

1. Carry out isoelectric focusing of granulocyte membranes in a tube gel in the first dimension and SDS-PAGE on a large gel for the second dimension (1,3).

3.2. SDS-PAGE

1. Electrophorese HeLa cell extract on 15% mini-gel SDS-PAGE as well as BSA on 10 as well as 7.5% mini-gels (1).

3.3. Flat bed native isoelectric focusing

1. Electrofocus rabbit anti-spectrin on flat-bed native IEF (0.4-mm gel thickness) (4).

3.4. Gel staining

1. Stain gels with 0.05% Coomassie brilliant blue (*see* Note 2).
2. Rinse with water (*see* Note 3).
3. Destain gel with destaining solution (*see* Note 4). Add a few sheets of Kimwipes to absorb the released Coomassie stain (*see* Note 5).

3.5. Gel storage in polyethylene bags

1. Incubate the destained gels in 50% methanol containing 5% glycerol for 30 minutes.
2. For a large, regular SDS-PAGE gel, follow the following protocol. Cut a polyethylene bag (originally open at one end) along one of its sides. Lift the top flap of the bag and place the SDS-PAGE gel on the lower flap. Lower the top flap slowly on top of the gel, such that no air bubbles are trapped (*see* Note 6).
3. Store the polyethylene bag at room temperature without heat sealing.
4. For a mini SDS-PAGE gel as well as the smaller native IEF gel, insert the gel into the bag through the open end of the bag without cutting the side of the bag. Store the gels at room temperature without sealing.

1. Notes

1. Weigh the required amount of the dye and dissolve it in methanol first. Filter the dissolved CBB through a circular Whatman 1M filter paper folded into a cone and fitted inside a glass funnel. For a quicker way to filter the CBB solution, layer a circular 1M Whatman filter paper (125 mm diameter circles) inside a Buchner funnel (Coors, USA; 135 mm inner diameter). The filter paper should cover all the holes in the funnel and lay flat inside the funnel. Attach the Buchner funnel to a side-arm fitted conical flask that is attached to the house-vacuum through the side-arm. Pour the CBB solution into the Buchner funnel and apply vacuum. If filter paper gets clogged, replace with a fresh one and filter. Collect the filtered CBB. Adjust volume with water, methanol and acetic acid to obtain a 0.05% CBB in 25% methanol/10% acetic acid.
2. Gels can be stained faster by heating the CBB staining solution. Place the gel in a container with the staining solution, with its lid in place. The container can be

heated in a shaking water bath maintained at 65°C for 20 minutes or in a microwave for a few minutes (9).

3. Rinse the CBB off the gel with few mL of ultrapure water prior to adding destaining solution. Do not discard the wash into the sink. Save the rinse in a bottle for proper disposal.
4. Gels can be destained faster by heating the destaining solution. Place the Coomassie stained gel in a container with the destaining solution, with its lid in place. The container can be heated in a shaking water bath maintained at 65°C for 20 minutes or in a microwave for a few minutes (9).
5. Kimwipes bind the released CBB very strongly. The Kimwipes do not release the bound CBB even after they were incubated in water for over five weeks or in high salt solutions (9). The Kimwipes with the bound CBB can thus be discarded as solid waste. This serves as an environmentally safe way to discard CBB and enable the destain to be recycled (if the gel did not contain radioisotopes).
6. Lower the top flap slowly on the gel, starting from one side of the gel, to cover the entire gel. The thin film of water on either side of the gel enables the polyethylene sheets to seal air tightly and thus provide a microsatuated atmosphere over both sides of the gel.

References

1. Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227, 680–685. [PubMed: 5432063]
2. O'Farrell PH (1975) High resolution two-dimensional electrophoresis of proteins. *J Biol Chem* 250, 4007–4021. [PubMed: 236308]
3. Sambrook J, Fritsch EF, and Maniatis T (1989) *Molecular Cloning*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
4. Kurien BT, and Scofield RH (1997) Multiple immunoblots after non-electrophoretic bidirectional transfer of a single SDS-PAGE gel with multiple antigens. *J Immunol Methods* 205, 91–94. [PubMed: 9236919]
5. Kurien BT, and Scofield RH (2009) Affinity immunoblotting for analysis of antibody clonotype distribution in a lupus patient developing anti-Ro 60 over time. *Methods Mol Biol* 536, 45–53. [PubMed: 19378044]
6. Kurien BT, and Scofield RH (1998) Long-term wet storage of sodium dodecyl sulfate-polyacrylamide gels in polyethylene bags. *Anal Biochem* 261, 116–118. [PubMed: 9683520]
7. Phelps DS (1984) Electrophoretic transfer of proteins from fixed and stained gels. *Anal Biochem* 141, 409–412. [PubMed: 6208814]
8. Kurien BT, Newland J, Paczkowski C, et al. (2000). Association of neutropenia in systemic lupus erythematosus (SLE) with anti-Ro and binding of an immunologically cross-reactive neutrophil membrane antigen. *Clin Exp Immunol* 120, 209–217. [PubMed: 10759785]
9. Kurien BT, and Scofield RH (1998) Heat mediated quick Coomassie blue protein staining and destaining of SDS-PAGE gels. *Indian J Biochem Biophys* 35, 385–389. [PubMed: 10412235]

Fig. 1.

(A) Two dimensional electrophoresis gel (1.5 mm thick) containing separated granulocyte membrane proteins at Day 1 of storage in a polyethylene bag. Granulocyte membrane proteins were first separated on the basis of charge in the first dimension and on the basis of size in the second dimension.

(B) The gel shown in (A) after storage in the polyethylene bag for over 2 months. The gel with the separated granulocyte membrane proteins was stored for over 2 months in the bag at room temperature.

Fig. 2:

(A) SDS-PAGE gel (10%) of BSA at Day 1 of storage in a polyethylene bag. Bovine serum albumin was electrophoresed, stained, destained, and stored. Lane 1- molecular weight standards. Lanes 2, 3, and 4 – 3, 6, and 12 mg of BSA, respectively. (B) The gel shown in (A) after storage in the polyethylene bag for over a month. The gel was stored at room temperature for over a month in the polyethylene bag.