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Note

Slab gel designed for enzymatic digestion of proteins in polyacrylamide gel slices and direct resolution of peptides

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The procedure of Cleveland *et al.*[1] is very useful in producing one-dimensional peptide maps for comparison of proteins separated by sodium dodecyl sulfate (SDS) electrophoresis. In this procedure a stained band is cut from an SDS gel, soaked in a buffer compatible with the proteolytic enzyme to be used and placed in the sample well of a second SDS gel along with the enzyme. Electrophoresis is begun immediately. Digestion occurs in an elongated stacking gel and resolution of the peptides occurs in the running gel.

I have used this system to compare peptide maps of proteins which can be resolved only by two-dimensional electrophoresis. In these instances it is desirable to maximize the amount of protein in each excised gel slice. Thus, thicker gels which contain more protein must be used. Conversely, peptides are better resolved in thin gels. I have therefore designed a simple and easy-to-use gel slab which features a thick well section above thin stacking and running gels.

In the procedure described by Cleveland et al.[1], protein bands are cut from the gels after a very brief time in stain. Such an approach is not feasible if protein spots or bands are present in low concentration and are narrowly separated. In these cases the gels must be completely stained and destained. This in no way changes the efficacy of the Cleveland *et al.*[1] procedure. Complete staining and destaining do, however, have two ramifications. First, the original gel buffer is completely exchanged. Thus, the proteins investigated can be separated by any system, *e.g.* acetic acid–urea gels[2], Triton gels[3], or CTAB gels[4].

Second, the gel pieces must be soaked longer in order to equilibrate them with the proper buffer[5]. This longer soaking causes the gel pieces to swell. Thus, the gel used for protein digestion and peptide resolution must have wells thicker than the original gel used for protein separation.

The gel slab shown in Figs. 1 and 2 features 1.5-mm thick stacking and running sections and a 3-mm thick well section. It is formed by using a second notched plate made of acrylic plastic 1.5 mm thick. By varying the thickness of the plastic notched plate, a well of any desired thickness may be achieved. The gel slab is designed to be run in a system similar to that of Studier[6] including the vertical slab gel systems marketed by Bio-Rad Labs. (Richmond, CA, U.S.A.). It can also be used with systems which do not require notched plates, *e.g.* the system of Matsudaira and Burgess[7] or the systems marketed by Pharmacia (Uppsala, Sweden).

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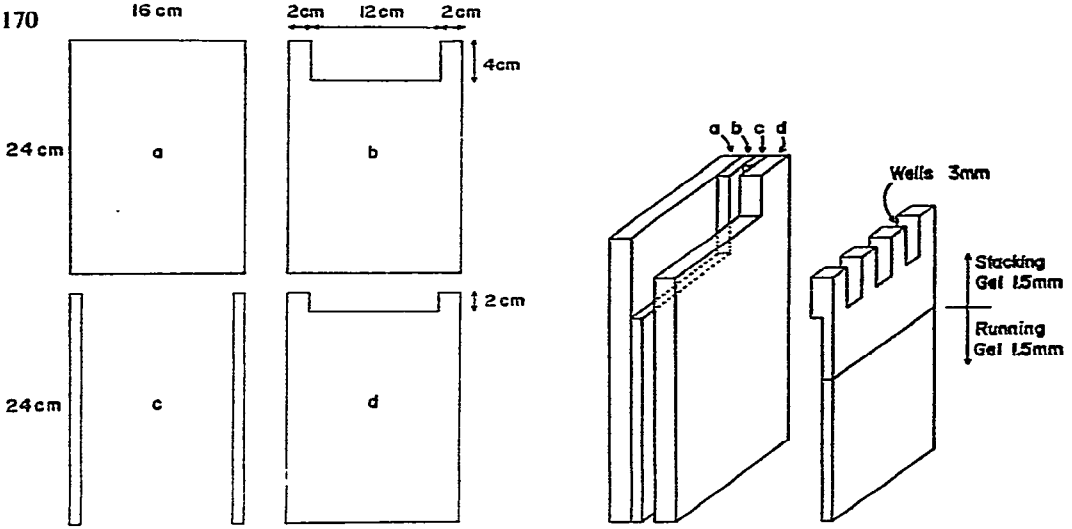

Fig. 1. Components of the slab gel mold. (a) Un-notched glass plate; (b) acrylic plastic plate with deep notch; (c) spacers; (d) notched glass plate.

Fig. 2. Perspective cross-sectional views of (left) assembled components from Fig. 1 and (right) polyacrylamide slab gel resulting from the use of the mold.

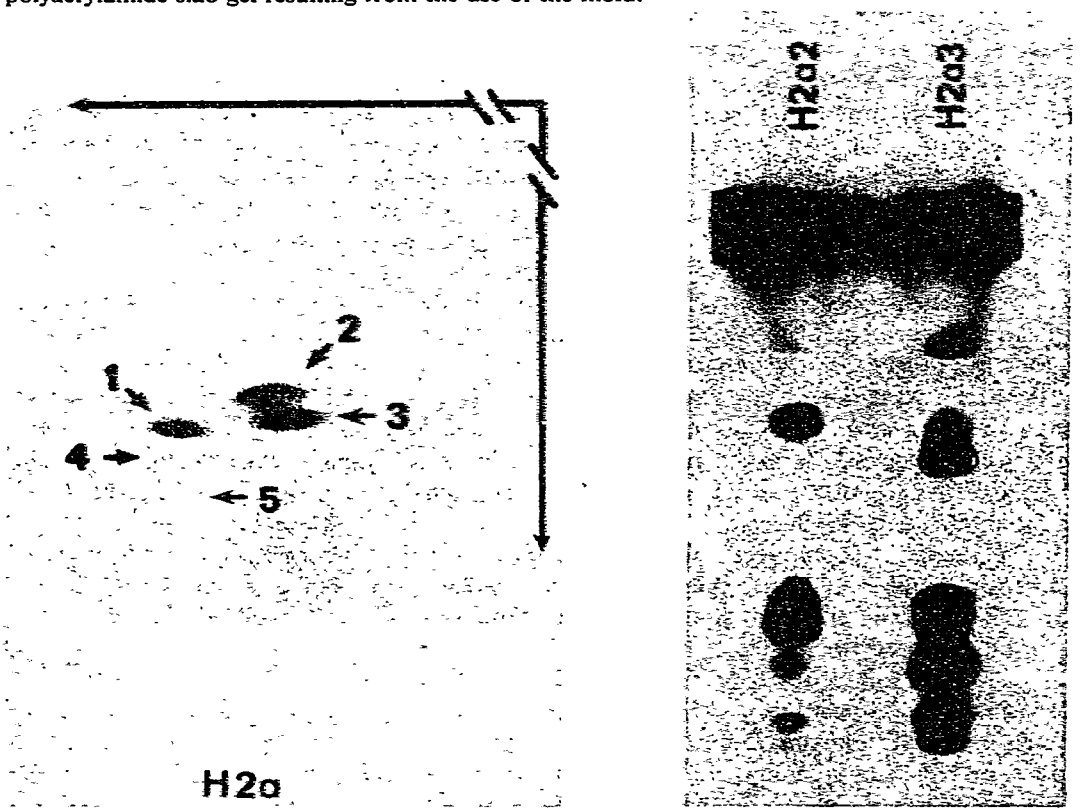

Fig. 3. Two-dimensional electrophoresis of wheat H2a histone variants. Gel was stained and destained. H2a2 and H2a3 spots were cut out and digested and the peptides resolved as shown in

Fig. 4. One-dimensional peptide maps of H2a2 and H2a3 from Fig. 3. Digestion was with *Staphylococcus aureus* V8 protease according to Cleveland *et al.*[4] as modified by Luna *et al.*[5]. The gel had a 3-mm well section and 1.5-mm stacking and running sections as shown in Fig. 2. Peptides were stained with silver according to Switzer *et al.*[8].

Measurements given in the figures can be modified to fit the dimensions of the glass plates in any existing slab gel apparatus. It is important that the comb be exactly equal to the thickness of the spacers plus the notched plastic plate. This ensures a snug fit for the comb between the glass plates. In the illustration, the notched plastic plate and spacers are cut from the same piece of acrylic plastic. The comb (not shown) is then cut from a double thickness (two sheets laminated together) of the same plastic.

Examples of peptide maps made by using this slab are shown in Fig. 4.

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