Preventing Horizontal Streaks on 2-D Gels

Haiyin Chang, Mary Grace Brubacher, and William Strong, Bio-Rad Laboratories, Inc., 2000 Alfred Nobel Drive, Hercules, CA 94547 USA

In troubleshooting any 2-D streaking problem it is important to keep in mind two things: 1) horizontal streaks result from incomplete protein focusing in the first dimension (isoelectric focusing, or IEF), and 2) vertical streaks result from poor protein separation in the second dimension (SDS-PAGE). Here we focus on causes and prevention of horizontal streaking.

Excessive Volt-Hours in the First Dimension

A longer focusing period does not necessarily improve resolution. In fact, it can cause horizontal streaks. Some protein samples are easier to focus than others, so a volt-hour optimization should be performed independently for each sample type with each buffer to determine the steady-state IEF pattern.

Incomplete Focusing in the First Dimension

Incomplete focusing will also result in horizontal streaks (Rabilloud 2000). Make sure the total volt-hours is appropriate for the length and pH range of the IPG strip being used. Focusing more than one sample type, or IPG strips of different pH ranges at the same time (i.e., in the same focusing tray), is often overlooked as a potential explanation for incomplete focusing of one of the samples. IEF cells set a total current limit for the whole tray. If one particular sample is more conductive, it will draw most of the current, slowing the focusing of the other protein samples in the tray. This leads to incomplete focusing and horizontal streaks. Therefore, samples with very different conductivities should be run separately.

Protein Overloading

The total amount of protein to load onto an IPG strip usually depends on the length of the strip and the stain that will be used to visualize the results (see table). Certain circumstances require additional consideration, such as when particular proteins constitute a large percentage of the total protein. This is the case with serum albumin, which makes up 70–90% of total serum protein. Thus, loading the recommended amount of protein likely would not show many other proteins, because they are masked by albumin. Under these circumstances, resist the urge to compensate by loading more protein — this could worsen the horizontal streaking. Instead, remove the serum albumin using Aurum™ Affi-Gel® Blue mini columns (catalog #732-6708) and then load the recommended amount of protein.

High protein loads, which result from loading too much protein into sample cups during cup loading, or from using IPG strips for preparative runs, can allow protein aggregation. This can result in protein precipitation at a particular protein's isoelectric point (pI fallout), which leads to horizontal streaking. Slightly increasing the IPG strip rehydration volume (~10%) may result in improved focusing. Suggestions described in the sections below for using reduction-alkylation and stronger detergents can also help to reduce protein-protein interactions and thus some of these problems.

Table. Guidelines for sample loading on IPG strips.

	IPG Strip Length				
	7 cm	**11 cm**	**17 cm**	**18 cm**	**24 cm**
Rehydration volume per strip	125 µl	185 µl	300 µl	315 µl	410 µl
Protein load					
Silver stain	5–20 µg	20–50 µg	50–80 µg	50–80 µg	80–150 µg
Coomassie G-250	50–100 µg	100–200 µg	200–400 µg	200–400 µg	400–800 µg

Sample Preparation Problems

Any contaminant with a net ionic charge will affect IEF and lead to horizontal streaking (Rabilloud 2000). Common contaminants include salt, detergents (see figure), peptides, nucleic acids, lipids, and phenolic compounds. Ionic contaminants are also evident when the target current for IEF is reached very early in the run at very low voltage (the default value for the PROTEAN® IEF cell is 50 µA/strip). Lower voltages lead to prolonged IEF runs and horizontal streaking if the set number of volt-hours is insufficient to remove the ionic contaminants as well as achieve equilibrium focusing of the proteins. Sometimes this can lead to excessive IPG strip heating or burning of the strip. The easiest way to fix the problem is to reduce the ionic strength of the protein samples. If you know the problem is salt in the sample, try desalting columns or dialysis. Bio-Rad's ReadyPrep™ 2-D cleanup kit (catalog #163-2130) can remove most ionic contaminants, in addition to salts.

Certain charged detergents, such as SDS, can coat proteins and drastically alter their net charge, ultimately affecting their isoelectric point and leading to streaking and loss of sample from the IPG strip.

Nucleic acid contamination of samples also can contribute to horizontal streaking (Görg 1997). Many proteins bind nucleic acids, which can generate a mixed population of protein-nucleic acid species, each with distinct isoelectric points. Nucleic acids can be removed from samples by treating the sample with nucleases prior to IEF (Garfin and Heerdt 2000). Alternatively, nucleic acids can be removed by ultracentrifugation in the presence of spermine (Rabilloud 1996).

Polysaccharides that contain sialic acid and are negatively charged can produce similar horizontal streaking artifacts to those seen with nucleic acids. Uncharged polysaccharides can also cause streaking by blocking the pores of the gel, preventing sample entry into the IPG strip, and physically impeding focusing. Ultracentrifugation is often sufficient to remove carbohydrates.

In addition, carbamylation of polypeptides can generate charge heterogeneity in individual proteins, and although discrete trains of spots are often formed, under certain conditions — such as when broad pH gradients are used — smearing between spots can occur. Protein samples should be prepared using high-purity urea and not heated above 30°C.

The last cause of horizontal streaking related to sample preparation is disulfide bond formation (Walsh 1998). When disulfide bonds occur in a protein sample prior to 2-D, they occur randomly intra- and intermolecularly. Disulfide bond formation creates various protein configurations, including minor pI variants (appearing on a 2-D gel as a widening of a single protein spot) and multimers of the same protein (appearing as streaking tails on spots, phantom spots, missing spots, and smearing). Preventing disulfide bond formation eliminates these artifacts. Disulfide bond artifacts are particularly problematic in two instances: 1) analysis of basic proteins, and 2) use of longer IPG strips. Horizontal streaking due to disulfide bond formation can be reduced using the ReadyPrep reduction-alkylation kit (catalog #163-2090) in conjunction with cup loading of IPG strips (Bio-Rad bulletin 4006216).

Poor Protein Solubilization

Failure to completely solubilize all the proteins in a sample will result in horizontal streaking. The main components of standard sample buffers used in 2-D electrophoresis to maintain sample solubility are urea, a nonionic detergent such as Nonidet P-40 (NP-40) or a zwitterionic detergent such as CHAPS, and a reducing agent such as DTT. Other compounds can be included in this mixture, for example, thiourea (Rabilloud 1998) or novel detergents, especially those in the amido sulfobetaine family, such as ASB-14 (Rabilloud 1996), to improve the solubilization of membrane proteins. The combined effect of these reagents is to reduce horizontal streaking by better solvating the hydrophobic regions on the proteins and diminishing aggregation due either to hydrogen bonding among proteins or to interactions with the IPG gel matrix. Finally, it is always good practice to remove insoluble protein complexes by centrifugation prior to IEF to prevent them from interfering with the entry of other proteins into the gel matrix.

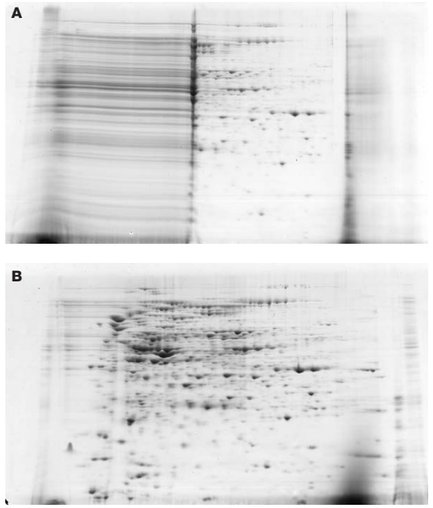

Figure. Example of horizontal streaking and its prevention. Detergent was effectively removed by the ReadyPrep 2-D cleanup kit, leaidng to improved 2-D SDS-PAGE separation. *E. coli* extracts were spiked with 1% SDS. A, untreated sample; B, sample treated with the ReadyPrep 2-D cleanup kit.

Electroendoosmotic Flow

Mainly a problem during IEF of basic proteins, electroendoosmotic flow can contribute to horizontal streaking due to water transport from the cathode to the anode. Water flow can slow protein migration in the opposite direction, leading to incomplete focusing. In addition, dehydration of the IPG strip at the cathode can reduce the pore size of the gel, leading to slower protein migration and incomplete focusing. Dehydration can also result in protein aggregation, and thus horizontal streaking. Solutions to this problem include replacing the paper wick at the cathode with a fresh water-soaked wick and adding organic modifiers such as glycerol, isopropyl alcohol, or methylcellulose to the rehydration/sample solution (Görg 1997).

In the next issue of BioRadiations we will discuss prevention of vertical streaking on 2-D gels. For more information and for answers to other technical questions, contact us at **consult.bio-rad.com**

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