Troubleshooting Forum

Molecular Biology Techniques Q&A

Native PAGE

This month's question from the Molecular Biology Forums (online at molecularbiology.forums.biotechniques.com) comes from the "Protein Methods" section. Entries have been edited for concision and clarity. Mentions of specific products and manufacturers have been retained from the original posts, but do not represent endorsements by, or the opinions of, *BioTechniques*.

What causes a purified protein to show up as a streak in native PAGE? (Thread 31053)

Q I purified a protein by chromatography and loaded it into a native PAGE gel. When I looked at the results, I did not see a crisp band, but instead, a large streak. I ran serum albumin on the same native PAGE and saw three defined bands at ~66 kDa, between 146 and 242 kDa (possibly a dimer of ~140 kDa that is a bit retarded relative to the monomer), and between 242 and 480 kDa (a trimer or tetramer). I used a native PAGE specific ladder, which indicated that the albumin monomer was at the expected size. My protein of interest, however, is ~40 kDa, but it forms a streak between 146 and 242. What could account for this?

A You are probably seeing aggregation of your protein of interest. You may be able to avoid this by adding components of the protein's storage buffer such as DTT, BME, or PEG to the gel and buffer.

Q I tried re-running the protein on an imidazole/tricine buffering system (as used in BN-PAGE, but without Coomassie G-250), and also tried using 0.1 or 0.5 mM DTT. I still see the same streak.

I placed an order for G-250 Coomassie and will try BN-PAGE next week. Do you know if that might solve the aggregation issues? I used only a small amount of DTT because a previous titration experiment (which involved SDS-containing Laemmli and SDS-containing top buffer) showed that the internal disulfide bonds of the protein are completely reduced at between 0.1 and 0.5 mM DTT, although perhaps the native protein would be able to tolerate a greater concentration.

I think my solution might be to reduce the concentration of protein to a minimum, run it on BN-PAGE, and then silver stain it. Would that help? The other option would be analytical ultracentrifugation to determine at what concentration the aggregation processes start to take effect.

A I have run native gels with 2mM DTT and it did not cause dissociation of the subunits. I think the SDS is probably responsible for the sensitivity of your protein to the DTT.

What buffer do you use to store your protein? You may want to add those components to your gel.

A You could be seeing degradation or cleavage products of your protein. Is your protein isolated in the presence of protease inhibitors?

Q I will try a higher concentration of DTT and see how it works. It makes sense that SDS and heat would make the protein more susceptible to DTT.

Yes, the protein was isolated in the presence of inhibitors. I don't think the problem is degradation since SDS-PAGE gives me a clean band. I think that there is some sort of aggregation going on that doesn't occur in serum albumin at the same concentration.

Q After a few attempts, I got the BN-PAGE and silver staining to work. My protein is forming regular multimers at higher concentrations, but I already see some dimers and trimers forming at 50 ng in a well.

Q2 I have a problem when running native PAGE with purified beta galactosidase. The enzyme was in monomer or dimer forms after purification. I loaded about 5ug of purified protein, along with 2x loading dye, in a 7% gel with non-denaturing conditions at 25mA for 6 hours. Rather than getting a sharp band, I got a large streak and a U-shaped band. I expected to see bands for the monomer at ~66 kDa and the dimer at ~132kDa. I used the same protein ladder I use for SDS-PAGE and the apparatus is also used for SDS-PAGE. I don't know what is causing the problem. Is the protein concentration too high or is there a problem with the loading dye? Can anyone offer any suggestions for solving this problem?

A Is the loading dye also used for SDS-PAGE? If so, then you are adding SDS and reducing agent and are denaturing your sample.

A You are not using the correct protein ladder. The protein ladder for SDS-PAGE is not suitable for native page. You need to use a protein ladder that does not contain SDS and reducing agent. Remember that charge is more important than size in native PAGE.

The U-shaped bands may be caused by diffusion of the sample or a wall effect against the teeth of the well.

Smears are often caused by incomplete solubility of the protein. You may be able to eliminate or reduce the smearing by clarifying the sample or ensuring that the pH is far enough away from the pI. You might also try finding a detergent (preferably non-ionic) that will completely solubilize the protein.

Q3 I have also encountered some problems when using native PAGE to separate protein bands. My protein is positively charged, so I'm considering increasing the pH of my sample buffer, running buffer, and gel buffer until the pH is greater than the pI so the protein will be negatively charged. I haven't tried this yet. Will it work or is there a better solution?

A What is the pH of the gels you are running? What is the pI of your protein?

The Ornstein and Davis native PAGE runs at a high pH. If you use anything much higher, you may interfere with polymerization. But if you are using a near neutral pH, then you can run higher pH gels or run acid pH gels and switch the electrodes.

Q I ran a continuous gel with the separating gel (15%) at pH 8.8. The pI of my protein is 8.83. So I was thinking of increasing the buffer pH to 9.0 or 8.9.

I previously tried switching the electrodes, but I didn't see any band.

A Your protein's pI is too close to the running gel pH, so you can't switch the electrodes.

You can run acid pH gels. There is a good formula in another post on this forum: <http://molecularbiologyforums.biotechniques.com/viewtopic.php?t=14433>

Q Actually, after staining the gel and treating it with methanol and acetic acid,

it looked like my protein was still in the well. It didn't mobilize at all. I used a sample that was eluted with a buffer including a high concentration of urea (8M). I wonder if that had an effect on the native PAGE. Could that be what caused the problem?

A No. For your protein of interest, simply reversing electrodes will not change anything. The pI is too close to the running pH, so your protein will be minimally charged, if even charged at all, and will not be significantly mobile. You will need to use a different PAGE formulation such as the one in the link I posted previously.

Is the urea still with the sample when it is applied to the gel? Urea will denature the protein. But Urea can be added to the gel to enhance charge separation.

A I agree with the previous post that your protein pI is too close to the running buffer pH. You can try lowering the pH to about 7 to see whether the mobility increases. If the mobility is still low, try an even lower pH, going down as far as 5 or 6.

I think you need to remove the urea before running native PAGE since it is meaningless to have urea in a native gel. When you do this, you may face another problem with precipitation of your protein. When you load a urea-denatured protein in a native gel, your protein may refold incorrectly and precipitate.

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