Molecular Biology Techniques Q&A

SDS-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 is the best way to separate proteins of similar molecular weight? (Thread 33111)

Q I need to separate 2 proteins with molecular weights of 86 kDa and 80 kDa; however, I haven’t obtained a good separation yet, even on a 6% polyacrylamide gel. To make matters worse, the two proteins are isoforms, and there are no commercially available antibodies against either one.

I’m considering using a 4% gel or maybe a 2-D gel, although both isoforms seem to have the same isoelectric point (pI). Can anyone recommend a better technique for separating these proteins?

A You’re going the wrong way with the gel concentration; you want to be more restrictive. Try a 10% polyacrylamide gel with a narrow range gradient, such as 10-12%. You might also add 4 M urea to the gel.

Q I tried a 4 M urea SDS-PAGE but saw fuzzy bands after probing. Could that be because I did not add urea to my samples? What amount of urea should I add?

A It is best if the urea concentration in the sample and gel is the same, but you can use a higher concentration in the sample if necessary.

Were your bands separated enough to distinguish between the isoforms?

Fuzzy bands can be the result of glycosylation, diffusion (from standing around too long before fixing or transfer, or soaking too long in the transfer buffer), or insufficient stacking, among other reasons.

Q I ran another 6 M urea, 10% polyacrylamide gel with 6 M urea-containing samples and saw rather compact bands; however, I think the fuzzy bands could be due to differences in protein expression levels. I’m using cells from lymphoid organs and cell lines with varying expression levels of my protein isoforms of interest. It looks like I will need to titrate the amount of protein loaded since the separation is still not clear.

Is it a good idea to let the dye front run off and only retain the protein of interest?

A That depends on what you want to show. If you are only interested in those two bands, then run the dye out of your gel. If you want reproducible results, run the gels for a specific number of volt-hours.

Q Since the separation is still not clear, does it make sense to use a higher percentage of urea?

A 8 M urea may improve the separation and make the bands sharper, but you may want to try a 12% gel, or even better, a 10-12% gradient gel.

Q Is a 30 min incubation of the samples in 8 M urea sufficient?

A Incubation with urea is not necessary. Also, be sure never to heat the urea; it will decompose and carbamylate your protein.

Q Even with these modifications, I see only minimal separation. One problem might be that I am loading too much protein on the gel. I will try reducing the input to reduce the thickness of the band.

A We saw improved resolution of our proteins using two electrode buffers instead of Laemmli buffer, along with running and stacking gel buffers.

A A longer gel with proper stacking or a gradient should work without too much band broadening, especially if you add some urea to the gel.

Selected by Kristie Nybo, Ph. D.

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