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**Supplementary Material**

**Identification and Characterization of Host Cell Protein Product-Associated  
Impurities in Monoclonal Antibody Bioprocessing**

**Nicholas E. Levy, Kristin N. Valente, Leila H. Choe, Kelvin H. Lee, Abraham M. Lenhoff**

### Quantification of missing spots from 2DE images

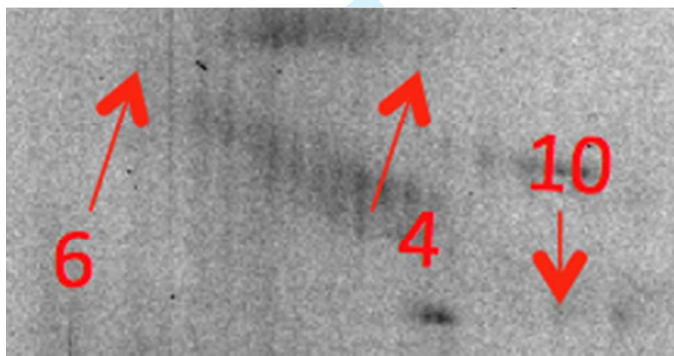
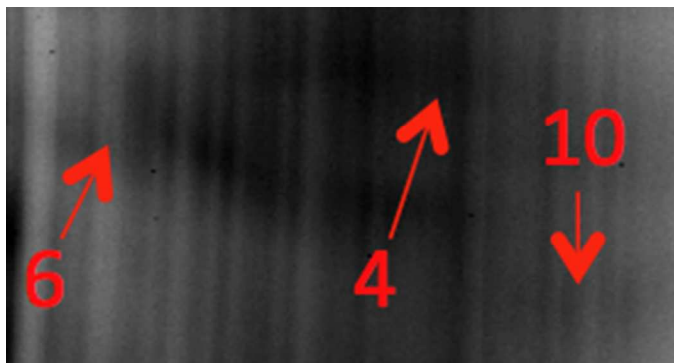
As described in the Methods section, ‘flow-through’ gels were compared to ‘load’ gels using Image Master 5 software. Spots that were found on the ‘load’ gel and missing from the ‘flow-through’ gel were identified as CHO HCPs associated with the immobilized mAb or mAb fragment. Using Image Master 5, the spots were integrated. The values in the table below reflect percent volume in flow-through divided by percent volume in load for spots of interest. A 50% reduction in spot volume was considered indicative of an associated HCP. For a few cases, due to either an obstructing artifact or very low total protein content in the flow-through gel, spot quantification did not indicate a 50% reduction in spot volume even though there was a qualitative change based on visual inspection. Enlarged images of ‘load’ and ‘flow-through’ spots of interest can be found below.

| Protein ID                                   | mAb A | mAb B | mAb C | mAb D | Fab D | Fc D |
|--|-------|-------|-------|-------|-------|------|
| 1. Neural cell adhesion molecule             | -     | *     | 0.14  | 0.32  | -     | -    |
| 2. Renin receptor                            | -     | *     | -     | 0.08  | 0.07  | -    |
| 3. Lipoprotein lipase                        | 0.38  | 0.09  | 0.45  | -     | -     | 0.34 |
| 4. Chondroitin sulfate proteoglycan 4        | *     | -     | 0.48  | 0.31  | 0.48  | -    |
| 5. Alpha-enolase                             | -     | 0.31  | -     | -     | -     | -    |
| 6. Galectin-3-binding protein                | 0.20  | -     | -     | 0.21  | -     | 0.26 |
| 7. G-protein coupled receptor 56             | *     | -     | 0.32  | 0.24  | -     | 0.25 |
| 8. V-type proton ATPase subunit S1           | -     | 0.32  | -     | -     | -     | -    |
| 9. Nidogen-1                                 | 0.40  | 0.10  | 0.18  | 0.29  | -     | 0.13 |
| 10. ATP synthase subunit beta, mitochondrial | 0.20  | 0.29  | -     | -     | -     | 0.26 |
| 11. Vimentin                                 | *     | 0.51  | -     | -     | -     | -    |
| 12. Heat shock protein                       | 0.16  | 0.45  | -     | -     | -     | -    |
| 13. Actin                                    | 0.10  | 0.27  | -     | 0.35  | -     | 0.35 |
| 14. Peroxiredoxin 1                          | -     | -     | -     | -     | -     | 0.17 |
| 15. a,b SPARC                                | 0.16  | 0.36  | 0.25  | 0.27  | *     | -    |
| 16. a,b Clusterin                            | *     | *     | 0.38  | 0.27  | *     | -    |
| 17. Complement C1r-a sub-component           | 0.37  | -     | -     | -     | -     | -    |
| 18. Metalloproteinase inhibitor 1            | 0.33  | -     | -     | -     | -     | -    |
| 19. Insulin                                  | 0.21  | 0.38  | 0.16  | 0.17  | -     | -    |
| 20. Cathepsin D                              | -     | *     | -     | -     | -     | -    |
| 21. Sulfated glycoprotein 1                  | 0.16  | -     | -     | -     | -     | -    |
| 22. Lysosomal protective protein             | -     | -     | -     | -     | -     | 0.38 |

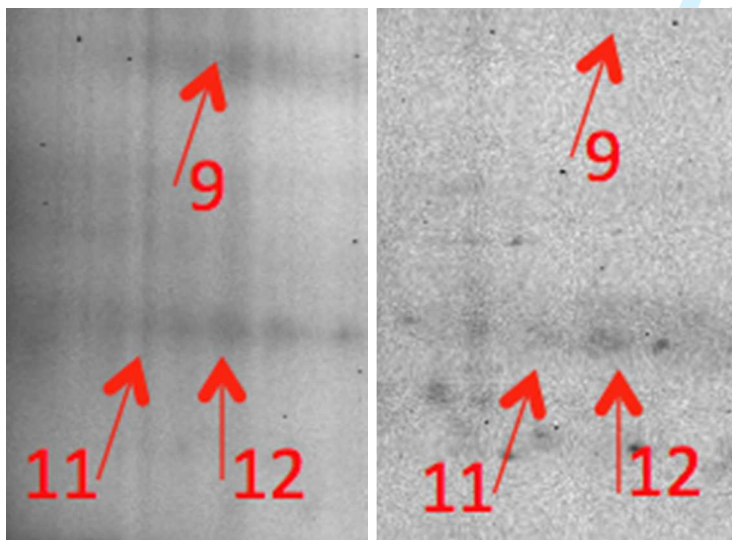
\* Cases for which spot integration did not confirm spots identified as missing based on visual inspection

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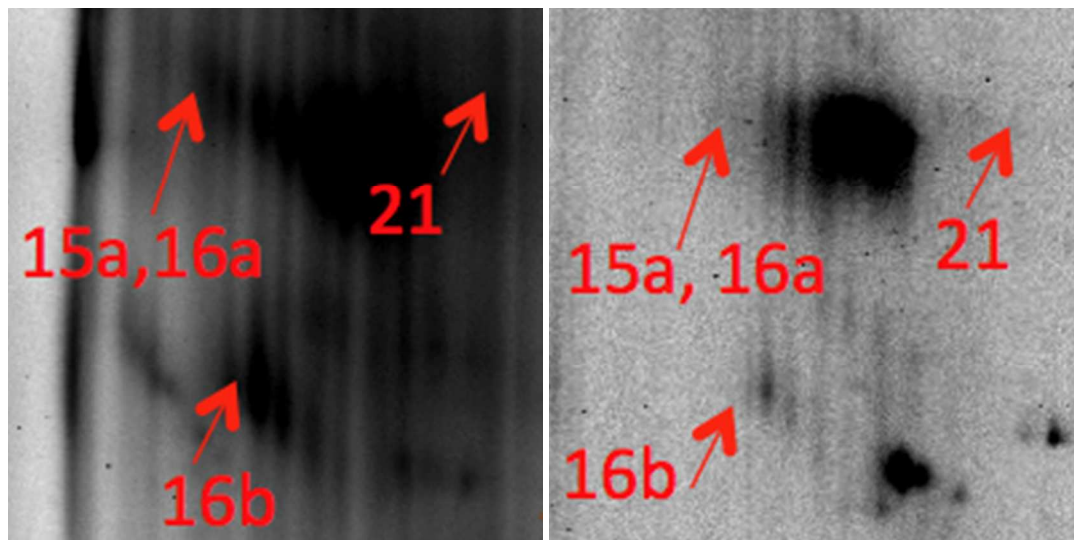
mAb A 'load' gel and 'flow-through' gel. Numbers refer to proteins in table above.



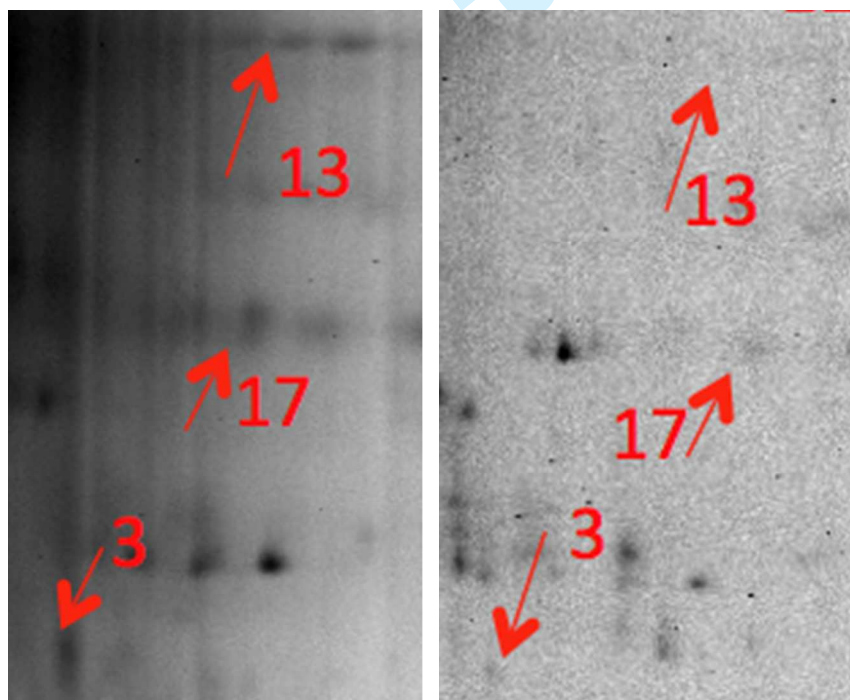
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Load (left) and flow-through (right)

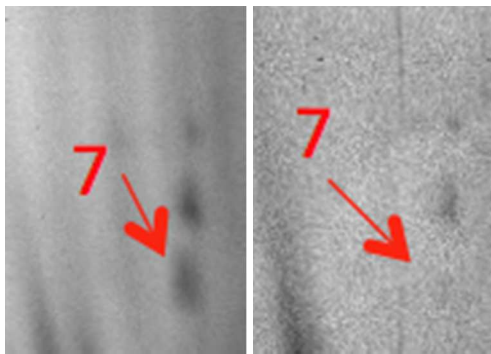


Load (left) and flow-through (right)

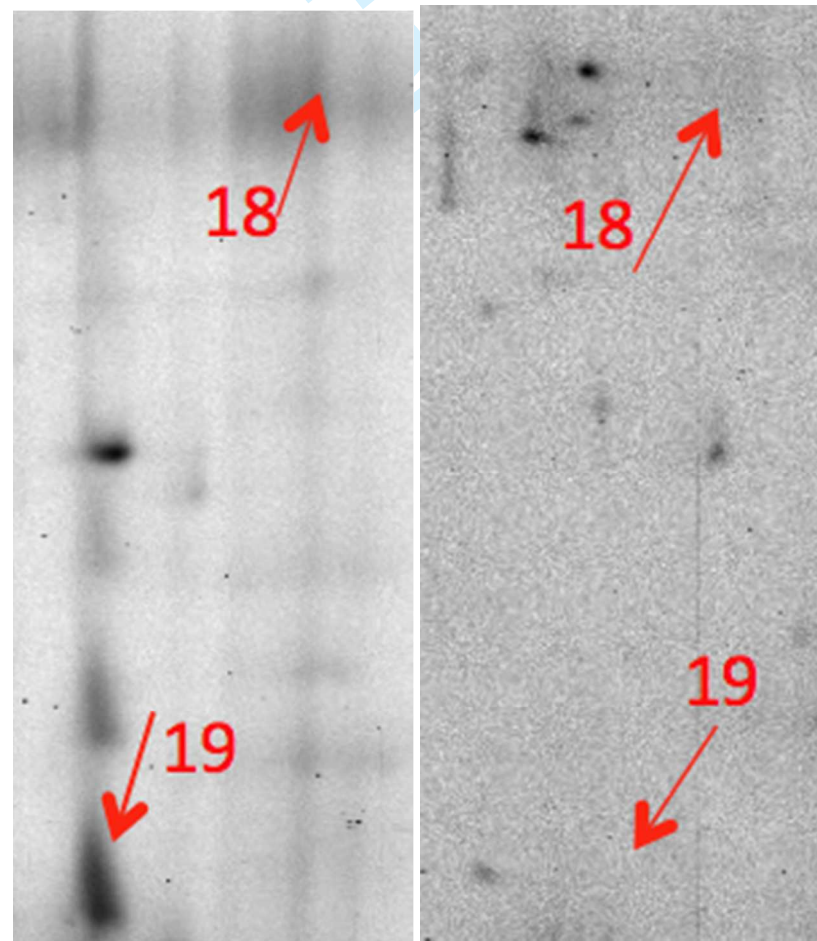


Load (left) and flow-through (right)

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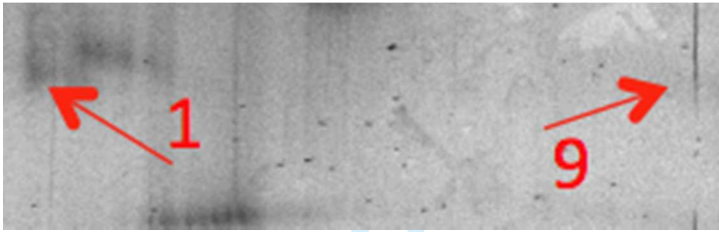
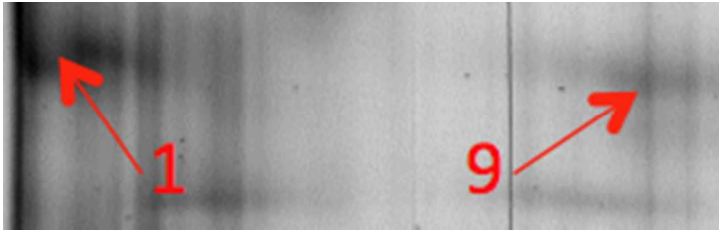


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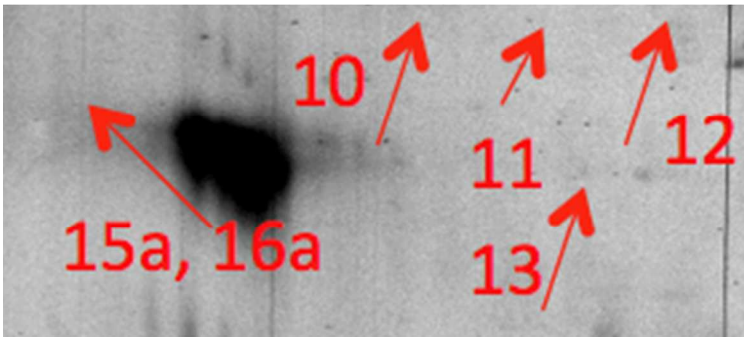
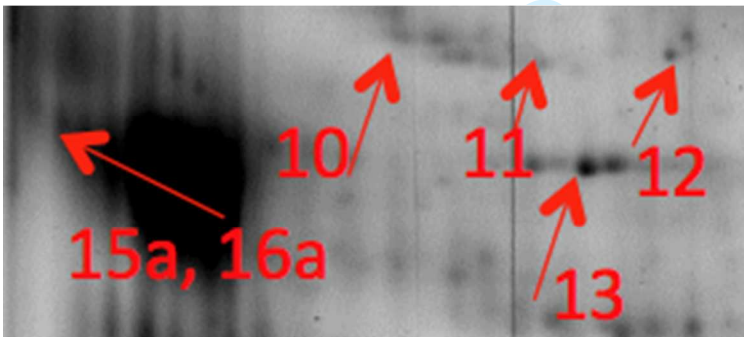


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mAb B 'load' gel and 'flow-through' gel. Numbers refer to proteins in table above.

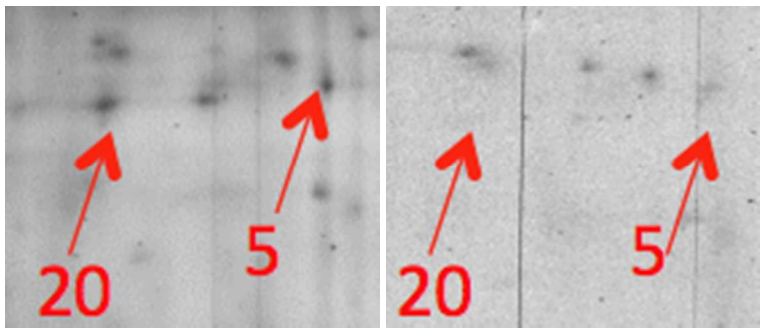


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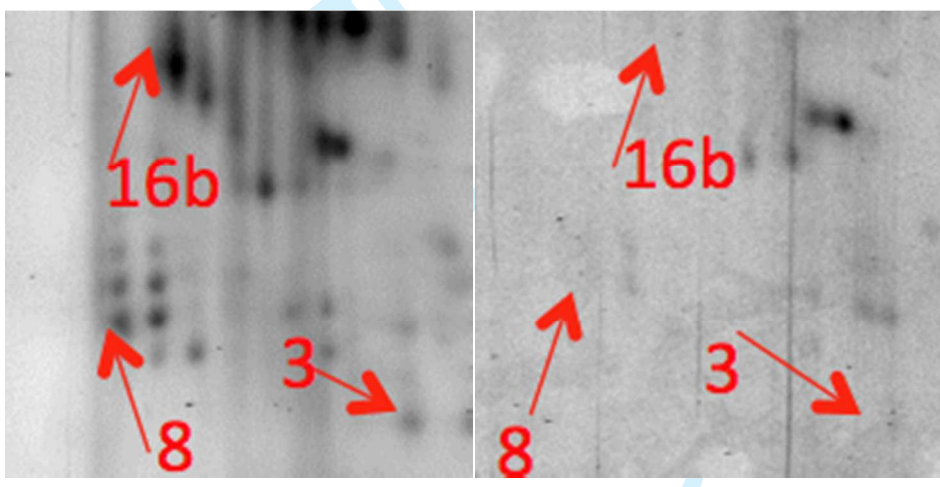


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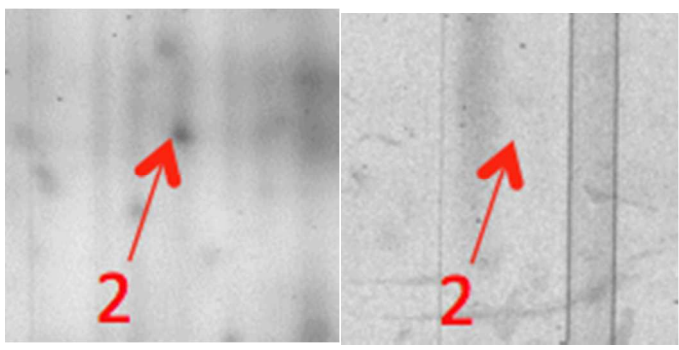
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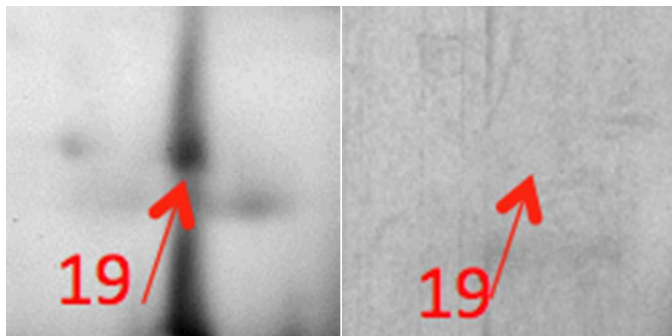


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Load (left) and flow-through (right)

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Load (left) and flow-through (right)

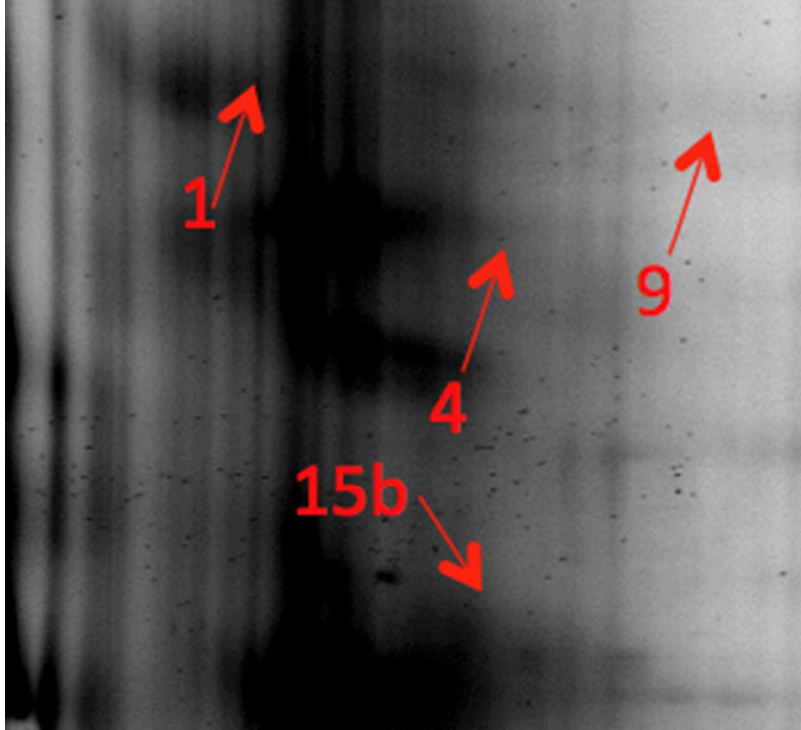
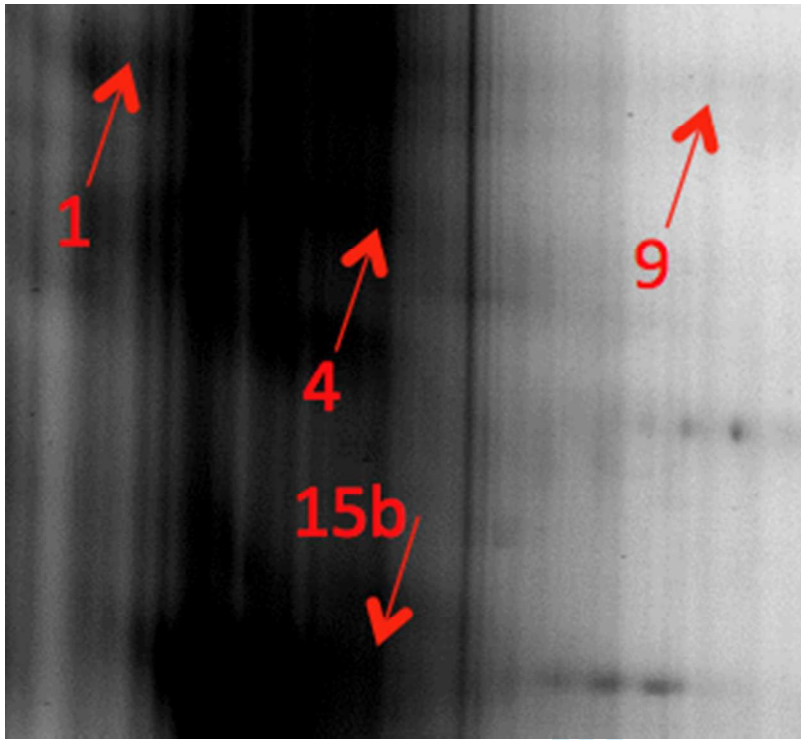
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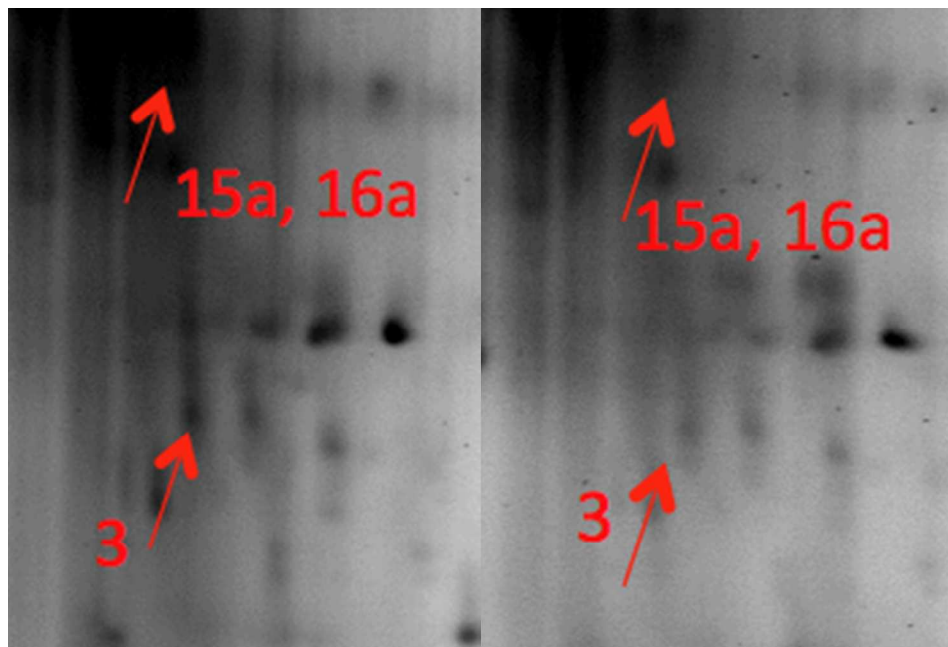


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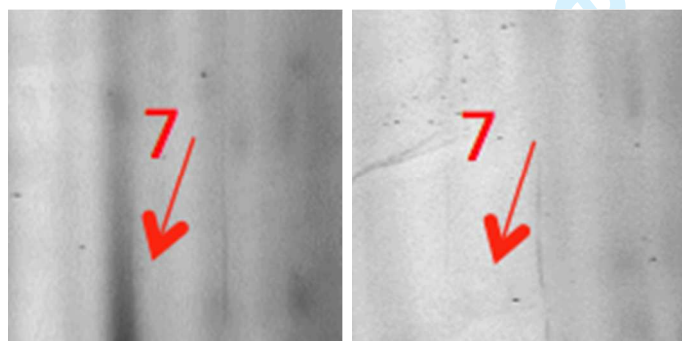
mAb C 'load' gel and 'flow-through' gel. Numbers refer to proteins in table above.



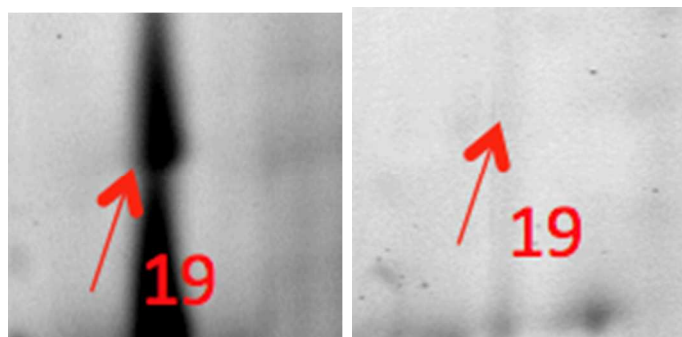
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Load (left) and flow-through (right)



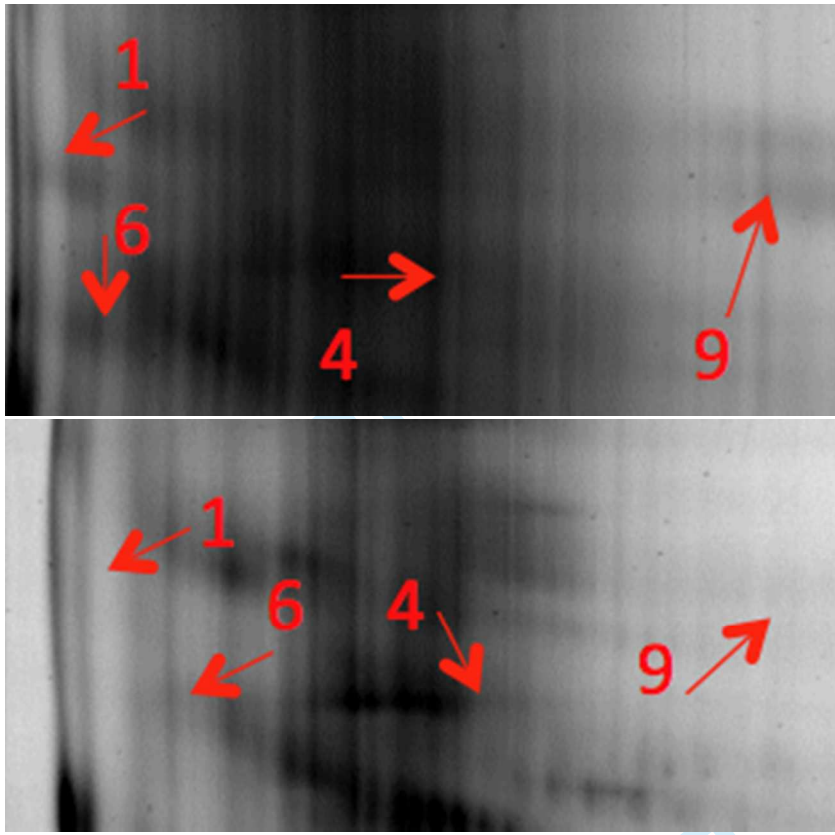
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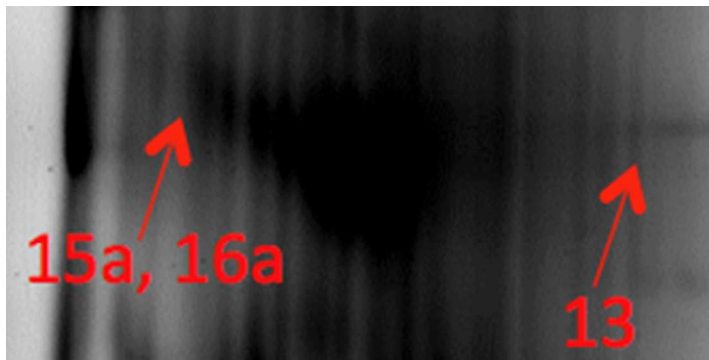
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mAb D 'load' gel and 'flow-through' gel. Numbers refer to proteins in table above.



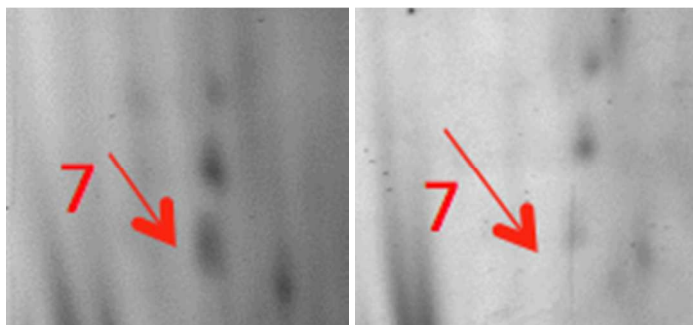
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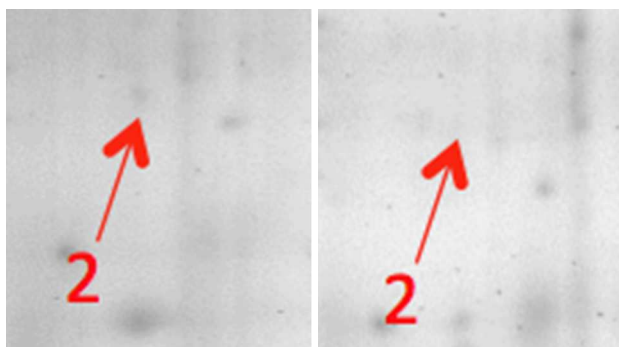
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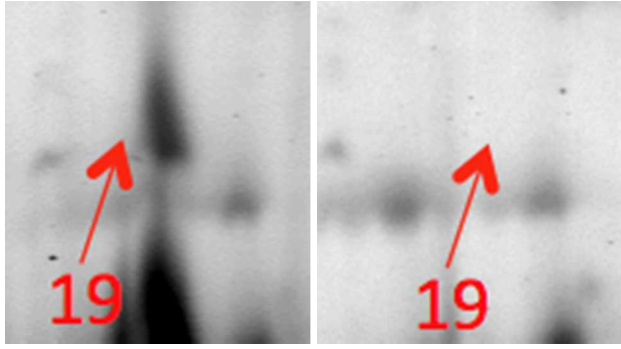
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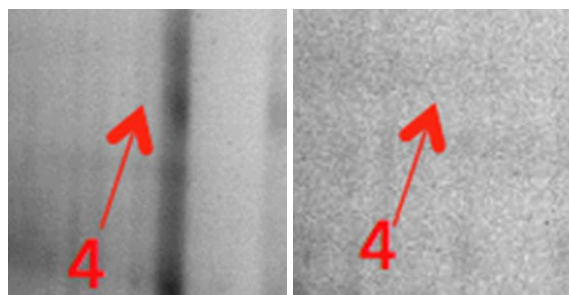
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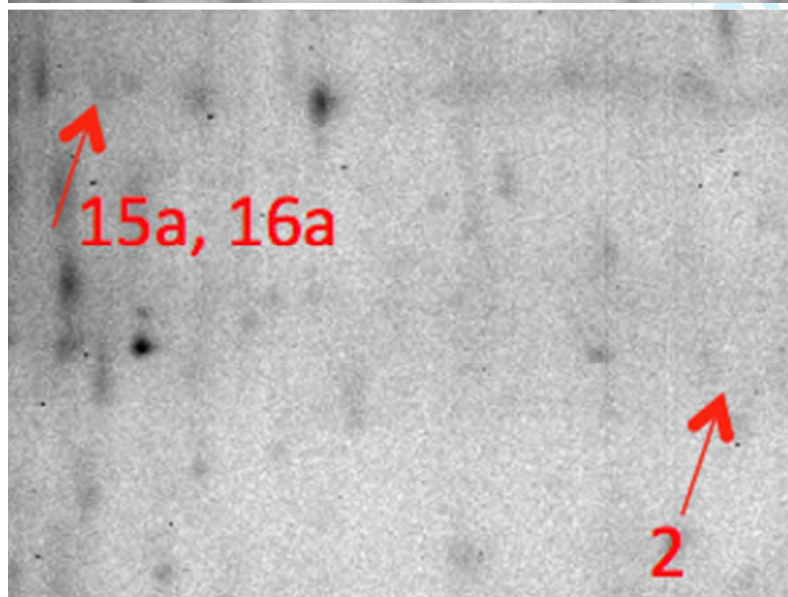
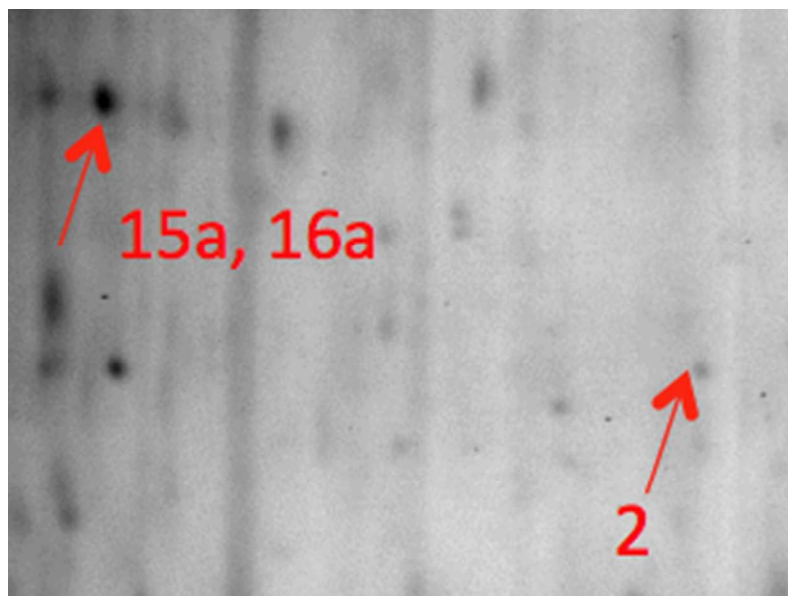
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Fab D 'load' gel and 'flow-through' gel. Numbers refer to proteins in table above.

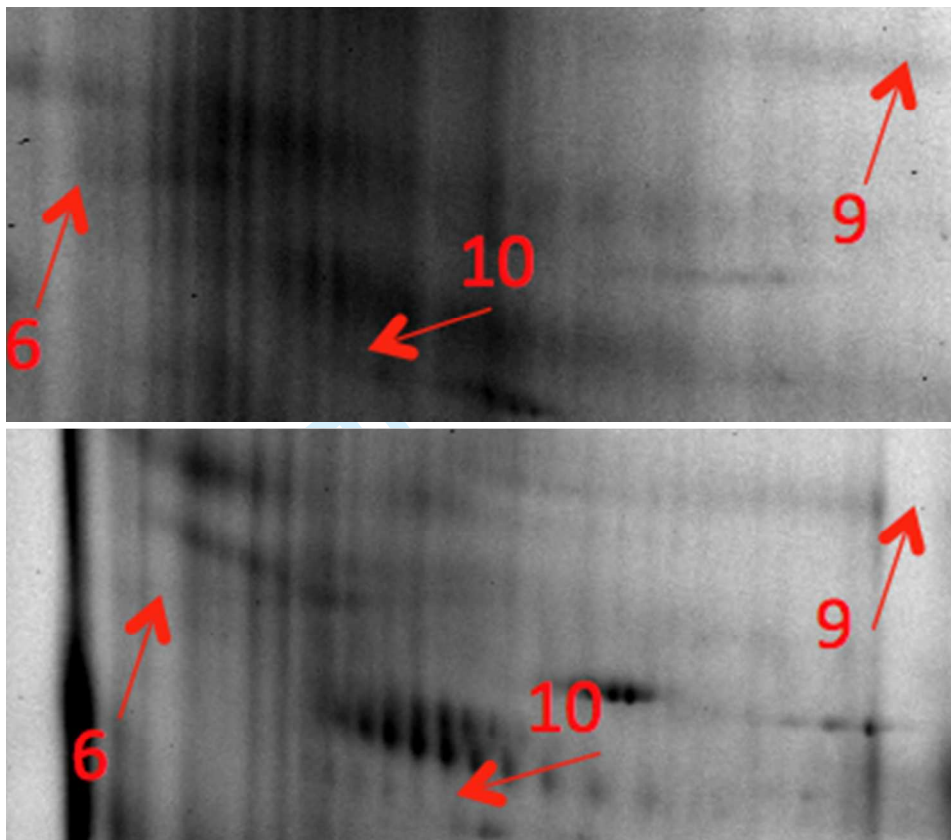


Load (left) and flow-through (right)

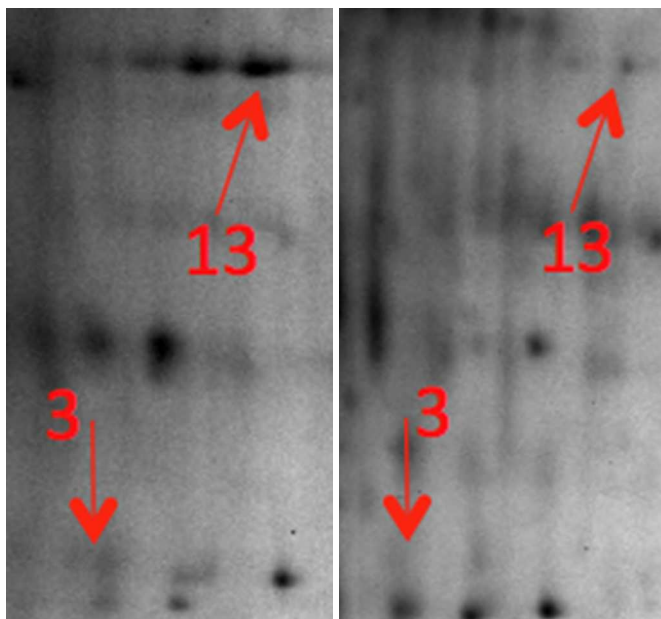


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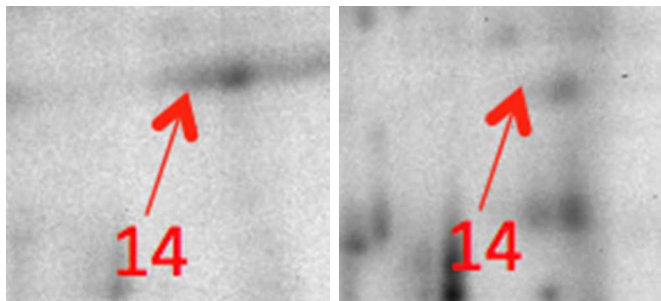
Fc D 'load' gel and 'flow-through' gel. Numbers refer to proteins in table above.



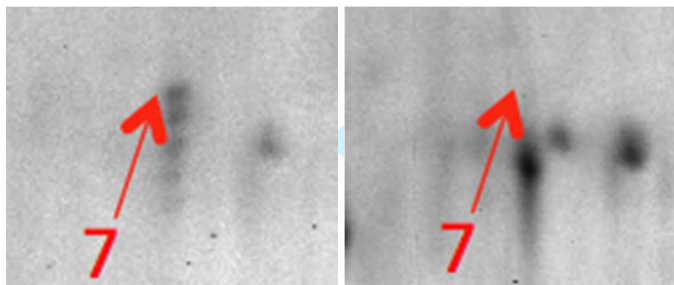
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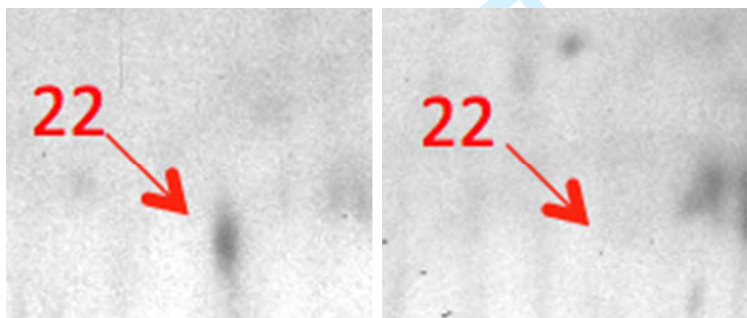
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