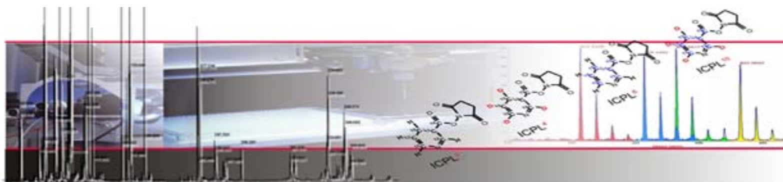


SERVA Electrophoresis

Frequently Asked Questions about the ICPL Method

1. **What lysis buffer can be used for ICPL sample preparation?**

   If lysis buffers different from the provided lysis buffer are used, they should be tested with the protein standard mixture before usage for other samples. Use pH 8.5; avoid the presence of primary amino group containing agents like tris buffers, ethanolamine, prim. ammonium salts (TRIS), AMP [2-amino-2-methyl-1-propanol], AMPD [2-amino-2-methyl-1,3-propanediol]. They will interfere with the ICPL reagents.Detergents like urea, thiourea, CHAPS can be used, however.

---

2. **What is the minimum protein concentration for ICPL analysis?**

   The protein concentration should be above 5 mg/ml, but must be in any case higher than 2.5 mg/ml. The protocol is optimized for a protein concentration of 5 mg/ml. However, it works as well at 2.5 mg/ml. As the recovery rate of the protein precipitation step depends strongly on the total protein concentration, losses are likely when working with lower protein concentrations. Therefore, it is extremely important, to keep strictly the concentrations of the reagents as recommended. If you want to work with increased sample volumes of 40 µl (for example to facilitate the pH measurement), you rather double the sample amount (if feasible) than dilute the sample, and you also have to double the volumes of the reagents given in this protocol! Therefore, only half the numbers of samples can then be labelled with the content of ICPL kit.

---

3. **What must be considered when adjusting the pH before ICPL labelling?**

   A pH-meter with a micro electrode is recommended. Before each measurement, the tip of the electrode is rinsed with distilled water and dried **very carefully** with a dry, dust-free tissue to avoid sample dilution.

4. **What are the optimal storage conditions for the ICPL Reagents?**

   For further storage overlay all ICPL Reagents with argon.

5. **How does the ICPL labelling influence subsequent 2D electrophoresis?**

   Please note for subsequent analysis that the ICPL labelling influences both the protein mass as well as the isoelectric point. Therefore it is recommended to use for 2D analysis IPG strips with a pH range from 3.5 to 4.5, e.g. SERVA IPG *Blue*Strips (Cat. No. 43027; 24 cm), instead of 3 to 6.

6. **Why is the Endoproteinase Lys-C not suitable?**

   After the modification of lysine (K) residues by ICPL, lysine is protected against proteolytic digestion. Therefore, trypsin cleaves solely C-terminal of arginine (R), i.e. only the Arg-C activity remains. Endoproteinase Lys-C cannot be used at all!

7. **How stable are the Glu-C (SERVA Cat. No. 20986) and Trypsin (SERVA Cat. No. 37284) after reconstitution?**

   The dissolved enzymes are stable for at least 4 weeks at -20 °C.

8. **How many peptide ratios are necessary for reliable protein quantification?**

   Reliable protein quantification requires at least 5 peptide ratios for a reasonable statistical treatment including outlier detection. If too many proteins provide fewer peptides for quantification, the pre-fractionation efforts prior to LC-MS/MS may need to be enhanced or chromatographic or mass spectrometric methods to be improved. Also repetition of the experiment is a standard approach to improve data reliability.

9. During the sample preparation there is a step of covering the samples with argon to avoid methionine oxidation. Is it possible to skip this step without compromising the labeling?

To avoid oxidation during alkylation and labelling steps, we recommend using protective gas. We did not validate the reaction without gas.

10. After the labeling, is it mandatory to precipitate with acetone or is it possible to skip this step, to avoid loss of sample?

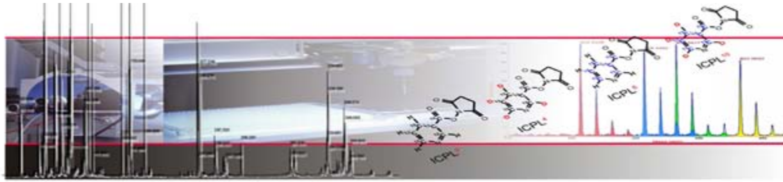
You have to get rid of excess labeling reagent and other stuff, so a concentration step is necessary. In our experiments acetone precipitation showed to have almost no loss of protein. Alternative methods could be ultrafiltration or dialysis. Please keep in mind: Labeling with ICPL affects the staining behaviour of proteins. Staining with silver is less intensive after labeling. The best results can be obtained by reverse staining with Zn-imidazole. (**SERVA*Snow* Staining Kit, Cat. No. 35080**; Fernandez-Patron et al. 1995).

11. After gel separation, is it possible to use ammonium bicarbonate as buffer for the digestion, instead of the suggested Tris?

In principle other buffers can be used. To achieve complete cleavage of proteins, it is necessary to keep the pH constant. The buffering capacity of the buffer used should be kept in mind. It is better for Tris.

12. The mass values to be inserted in mascot for the search are those reported in the attached technical note?

In new Mascot versions you can select ICPL. If you use an older version you have to insert the values given in the technical note.



13. What is the concentration of the ICPL- labels supplied in DMSO in your kits?

The concentration of each label is 150 mM in DMSO.

---

14. We are wondering if you have experimented with ICPL peptide labelling? If so, did you have to use a larger amount of label to compensate for the increase in labelling sites? We would like to continue to label on a peptide level with ICPL but we need to ensure as much labelling efficiency as what we see with standard ICPL labelling.

The concentrations of protein and ICPL reagent are critical during labeling and validated for the described values. If peptides are labeled the number of amino groups is increased by a factor of two in average and therefore also the amount of label has to be increased. We suggest, to alkylate the protein sample, precipitate with acetone, digest in a non amine containing buffer like TEAB and then performing the labeling step. Desalting and concentration can be done using ZipTips ore StageTips (RP C18).