

Gel Handling

The following table contains some helpful considerations to assure optimal results:

<p>Beware of Contaminations</p>	<p>Dust particles contain high levels of proteinaceous contaminations, e.g. keratines (hair and skin particles, fuzzy clothes) therefore carry out all staining steps in a closed dish, use chemicals and solutions dedicated to "mass spectrometry". Thoroughly wash anything that will come into contact with your sample (i.e. gel apparatus, staining trays, gel excising implements, gel storage equipment, and Eppendorf vials), filter solutions if necessary. Wear powder free gloves and lab coats at all times during sample preparation, cut the bands/spots under a laminar flow hood and do not touch the vials at any stage of the sample preparation without gloves.</p>
<p>Use Compatible Protein Staining Techniques</p>	<p>There are several methods applied to visualize proteins after gel electrophoresis. Unfortunately not all of them are compatible with the mass spectrometric analysis. Coomassie staining techniques are in general ideal for these investigations. However, there are some cases, when higher sensitivity is needed. Note, that silver staining applying glutaraldehyde in the sensitizing solution is not compatible with mass spectrometry! For this reason we recommend to use an alternative silver staining protocol (see Download Section). In case of silver staining do not overstain the gel, keep the background clear! According to our experiences, overstaining reduces the yield of identifiable peptides from the sample substantially! A sensitive as well as fully MS compatible detection of proteins can be achieved by the use of the fluorescent dye SYPRO Rubry.</p>
<p>Minimize Empty Gel Volume</p>	<p>Keep the volume of the empty gel matrix as small as possible, cut away unstained gel materials.</p>
<p>Fresh Sample</p>	<p>Submit the samples as soon as possible, freeze them only, if you can guarantee, that they do not thaw during transfer to our lab.</p>