IN-GEL SAMPLE PREPARATION GUIDELINES

The quality of sample extraction and preparation significantly impact MS results. For gel bands, please carefully follow the following instructions:

* CRITICAL: Special care must be taken to avoid contamination in every step, especially with keratins from skin or hair (always wears clean nitrile gloves and work in a dust- free environment).
* CRITICAL: DO NOT use silverstaining! Only Coomassie-stained gels!
* CRITICAL: PRECISELY cut out ONLY the band of interest (only the stained area) - any excess gel will lead to background noise. If the band is extremely faint, there is a likely chance that we won’t get any protein identification. So, we suggest you load the maximum possible amount of protein sample into the gel so that we get a fairly visible band with Coomassie stain.
* Email us a gel picture before sending the samples. This will help us to understand whether the band is properly stained or not. This could also be used as a reference to understand the sample concentration and its complexity. Since the nano-LC is a highly sensitive instrument, it is very important not to overload the nano-LC column with excess sample for the optimal results.
* ONLY cut gels on a clean glass plate (never use overhead projector foils or aluminium foils). The glass plate can be cleaned by using organic solvents like 70% ethanol or methanol.
* Use a NEW clean scalpel blade for precise cutting of gel spots.
* Always use filtered deionized water.
* DO NOT wash any flasks, tubes, or glass plates for electrophoresis with soap (or any polymeric detergent). Always, rinse your glassware with hot water and then an organic solvent like 70% ethanol or methanol.
* Freshly prepare all the buffers and stains needed to run the gel for MS analysis (DO NOT re-use).
* Place the gel cubes in a clean microcentrifuge tube (1.5 ml) and add enough methanol (50% methanol) in it to cover the gel cubes. The addition of large volumes of the buffer is not required, having them moist is enough.
* Make sure that the lid of the microcentrifuge tube is CLOSED PROPERLY before placing it in an envelope to send.
* DO NOT use parafilm around the lids.
* You can send the samples by courier or ordinary post. There is no need to ship the samples on ice. The samples are stable in 50% methanol.