

CHECK LIST FOR PREPARING GEL BANDS OR SPOTS FOR PROTEIN IDENTIFICATION BY MASS SPECTROMETRY

PLEASE NOTE:

- 1) NEVER dip into reagent bottles, and never use reagents or solutions used by all the lab.
- 2) Use sterile technique principles in preparing your samples, running gels and cutting out bands or spots.
- 3) Gel bands or spots from Invitrogen NuPage gels produce better signal to noise in the mass spectrometer than BioRad Criterion gels. Alternatively, cast homemade gels overnight.
- 4) Use natural color, non-coated tubes or plates
- 5) Whenever possible, use HPLC grade or better reagents and make solutions fresh.
- 6) Colloidal Coomassie Blue staining ([SimplyBlue](#) or [InstantBlue](#)) is preferred but any staining technique is acceptable if the technique is known to be mass spectrometry compatible. If you do not know if your technique is MS compatible check with the vendor or with us. If you use a silver stain kit, do NOT over stain the gel (longer than the protocol recommends), even if it is MS compatible.

_____ **Stain gel in clean container, washed glass or petri dish.**

Do NOT use plastic wrap or parafilm, they are good sources of keratin.

_____ **Capture an image of your gel to submit with your sample.** You may use our gel scanner to reduce the risk of contamination. Please contact us at 4-6968.

_____ **Cut PROTEIN band or large spots into 2 to 3 pieces.**
(pieces smaller than 1x1 mm will be sucked up into a pipet tip when dehydrated)

Cut out just the band or spot and the sooner the band or spot is cut out the better.

No extra unstained gel around the band or spot.

Stay away from the edges of the gel lane.

Large amounts of gel decreases efficiency of in-gel digestion and peptide extraction.

_____ **Place gel pieces into methanol or acetonitrile rinsed eppendorf tube or 96 well plate.**

If more than 8 samples, please put gel pieces in rinsed 96 well plate (www.bioexpress.com)

If we digest your samples, use BioExpress [T-3032-2](#), Greiner, V-bottom plates with [Costar 3080](#) storage mat III plate covers.

If you digest your samples, please submit dried digested samples in a rinsed 96 well

BioExpress [T-3084-1](#) plate with an BioExpress [T-2417-12](#) E-Pierce ZoneFree cover.

If you do not have access to these plates and covers, you can ask us for one.

Place samples in 96 well plates using the following order: A1, B1, C1, etc

_____ **Cut one BLANK control gel band or large spots into 2 to 3 mm pieces.**

No charge, used for trouble shooting gels.

_____ **Optional: Destain silver stained proteins immediately** ([Electrophoresis 20: 601-605 1999](#))

_____ **Wash gel pieces 2 times in 50% HPLC grade methanol or ethanol for 10 min each.**

_____ **Removed ALL of the excess liquid and store at -20°C.** Samples OK for months at -20°C.

_____ **Label tubes or 96 well plates with YOUR NAME, DATE and SAMPLE NAME(s).**