In-Gel Digestion Protocol

- Reagents required

1. Destaining Solution (250mM TEAB with 100% ACN -1:1)
2. LCMS grade Acetonitrile (100%)
3. 200 mM TEAB in LCMS grade water
4. Trypsin (MS Grade)
5. LCMS grade ACN with 0.1 % Formic Acid
6. 0.1% Formic Acid in LCMS grade water

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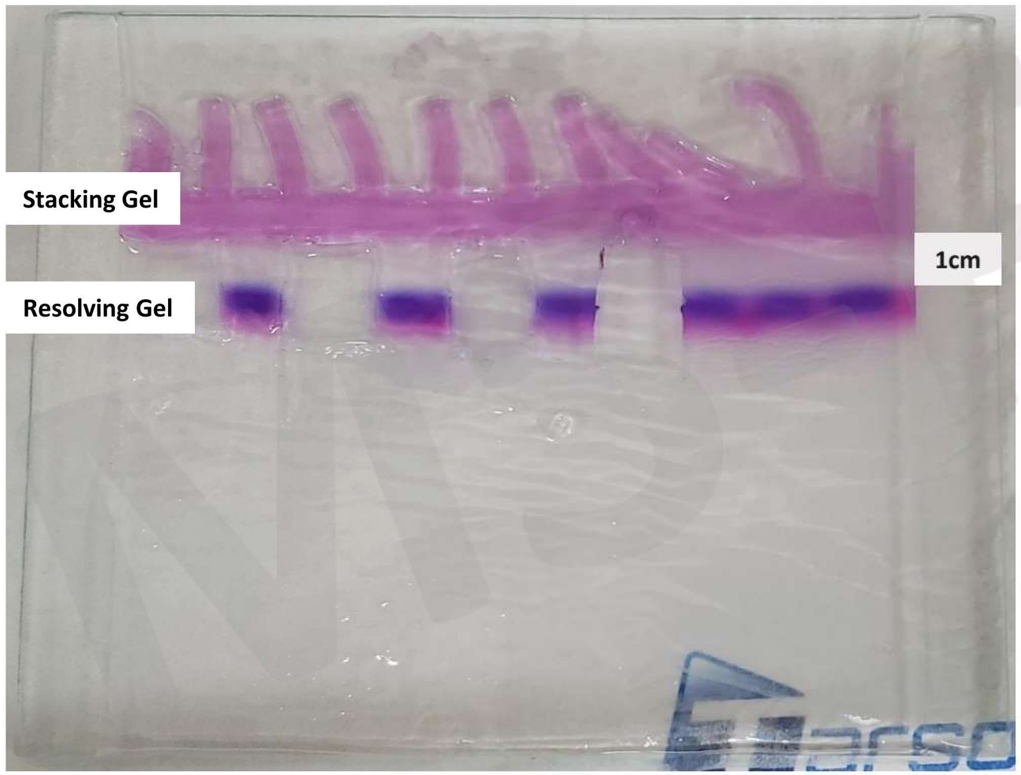
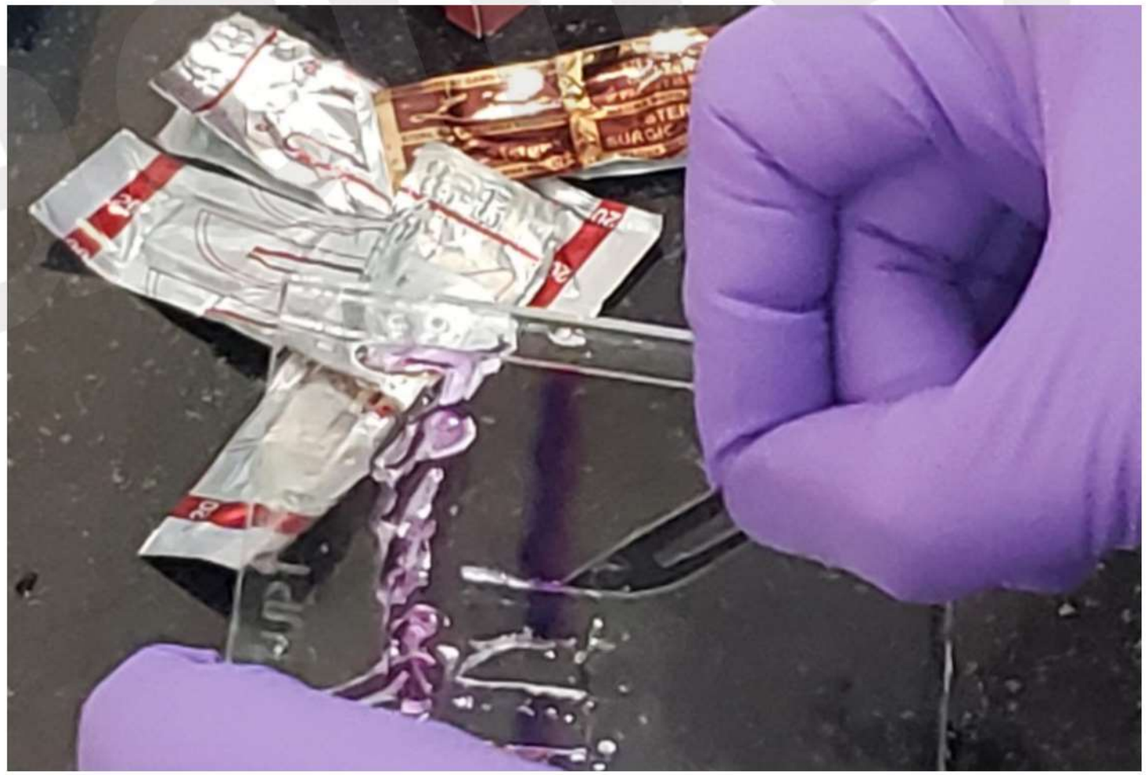
➢ SDS-PAGE gel for Immunoprecipitated and all other samples

1. Run the sample just 1-cm in the resolving gel. [**Do not resolve**]
2. Keep **empty lanes** between different samples



1. Cut the gel bands using clean surgical blades
2. Use **new blade** for each new band



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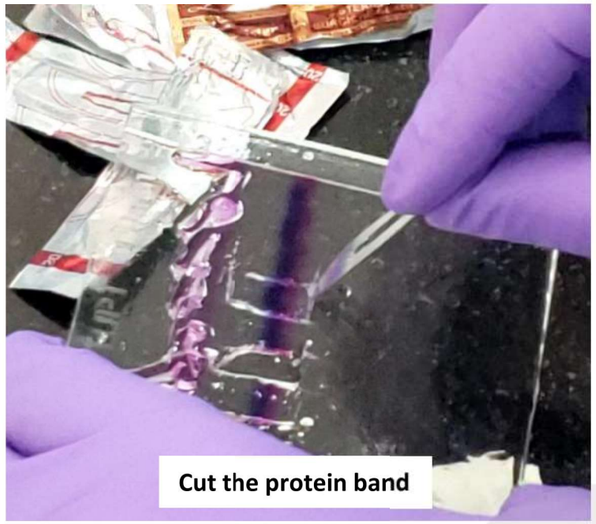
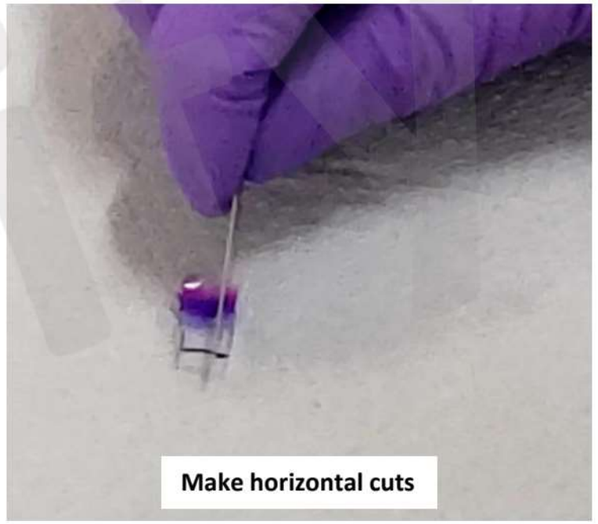
In-Gel Digestion Protocol

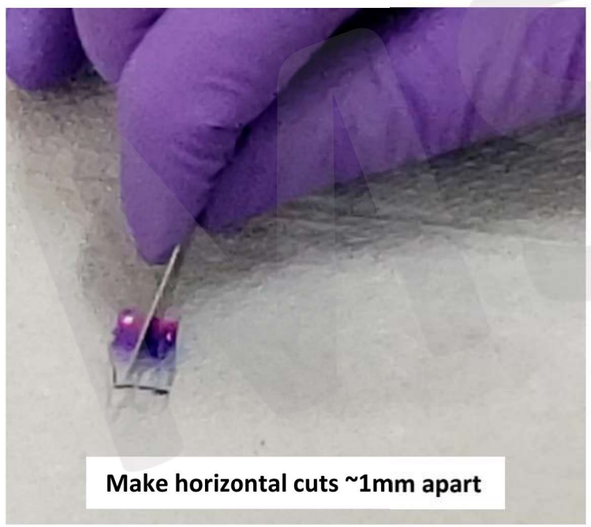
➢Cutting a gel band for digestion (make 1mm x 1mm pieces)

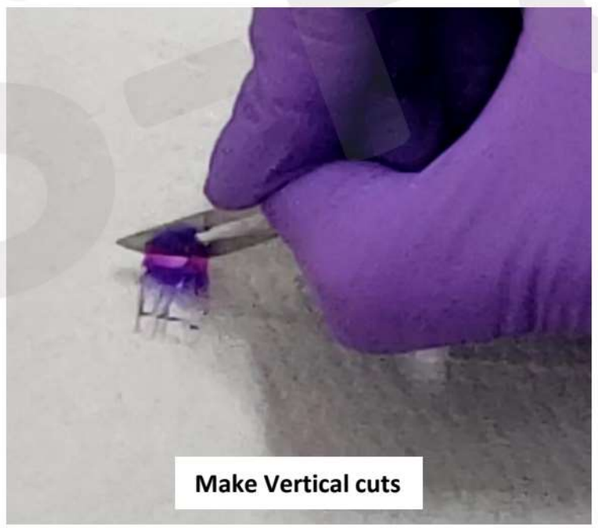

Cut the protein band

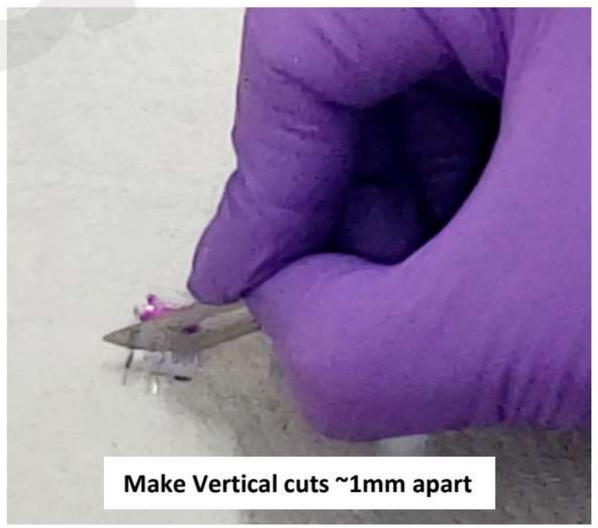

Lift the protein band


Put on clean glass slide


Make horizontal cuts


Make horizontal cuts ~1mm apart


Make Vertical cuts


Make Vertical cuts ~1mm apart


Put the gel pieces in a labelled tube

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In-Gel Digestion Protocol

➢ Sample Preparation

1. The gel pieces were washed with LCMS grade water thrice. Vortexed and the supernatant was discarded.
2. The gel pieces were chopped and destained with 1:1 250 mM Triethyl ammonium bicarbonate (TEAB) with 100% acetonitrile (ACN). Then supernatant was discarded.
3. About 200ul of 100% ACN was added and kept it in room temperature till the gel pieces shrunk (turns white). The supernatant was discarded
4. Repeat step 3 .
5. The gel pieces were dried for few minutes at RT.
6. Trypsin (1:50 :: Enzyme: Sample) with 100-200ul of 200mM TEAB (gel pieces should be completely inside TEAB) was added to gel pieces.
7. The samples were incubated on ice for 30 minutes.
8. The samples were kept for digestion at 37 °C for overnight.
9. 200ul of 100% ACN with 0.1% Formic acid was added to the sample and sonicated for 5 min. The supernatant was collected into a fresh tube.
10. Repeat step 8.
11. The supernatant was dried using Speed vac and reconstitute in 0.1% formic acid. Then desalting is done before injecting onto mass spec.

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