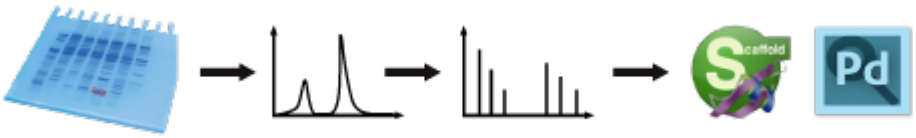
# In-Gel Digestion



## What is this method?

A digestion of proteins separated by gel electrophoresis.

## Advantages:

Robust, reproducible and effective method.

## Disadvantages:

Time consuming.

## Material:

- Criterion precast gels (Bio-Rad)
- Coomassie blue
- XT MOPS running buffer (Bio-Rad)
- XT sample buffer, XT reducing agent (Bio-Rad)
- Methanol
- De-stain solution (10% methanol/5% glac. acetic acid)
- Sterile scalpel
- Acetonitrile (ACN, 100%)
- Ammonium bicarbonate (AmBic)
- Tris(2-carboxyethyl) phosphine (TCEP) – make 20 mM TCEP in 50 mM ambic
- Iodoacetamide (IAA) – make fresh 200 mM IAA in 50 mM ambic
- MS grade Trypsin: 10 ng/µL (or another protease)
- Trifluoroacetic acid (TFA)
- Extraction buffer (EB): 50% ACN/0.1% TFA
- Formic acid (FA, 0.1%)
- Speedvac
- NanoDrop

## Procedure:

1. Mix 150 µL of XT sample buffer with 7.5 µL of XT reducing agent, add to a sample and boil for 5 min.
2. Load your sample to a gel and run electrophoresis.

3. Stain a gel with Coomassie blue for 2 hours and de-stain overnight. We currently use InstantBlue which stains gels in less than 20 min.
4. Take a picture of your gel, and cut gel pieces using sterile scalpel. You may decide to cut only specific bands, or cut the whole lane into sections if you are interested in seeing the whole proteome/interactome. Wash a scalpel with water, then methanol, then water, between cutting separate bands. Transfer gel cuts into eppendorf tubes and label.
5. You can stop here and store your gel cuts at -20 ºC or continue. Gel pieces stored in freezer are stable for several months.
6. Add 100 µL of HPLC-grade H2O, shake for 10 min, remove.
7. Add 100 µL of ACN, incubate for 5-10 min, remove.
8. For every sample mix 10 µL of 20 mM TCEP with 90 µL of AmBic (50 mM). Add 100 µL of TCEP solution to a sample and shake at 37 ºC for 30 min.
9. Add 100 µL of ACN and continue shaking for 15 min. If gel pieces are still blue, continue shaking for another 15 min, remove.
10. Add 100 µL of ACN, incubate 5 min, remove.
11. For every sample mix 10 µL of 200 mM IAA with 90 µL of AmBic (50 mM). Add 100 µL of IAA solution to a sample and shake at dark for 20 min, remove.
12. Add 100 µL of ACN, incubate 10 min, remove.
13. Add trypsin to cover gel cuts (20-50 µL) and incubate on ice for 30 min, remove excess trypsin.
14. Add 50-100 µL of 25 mM AmBic to gel cuts (it should cover it completely) and incubate overnight at 37 ºC. Check after 15 min if AmBic still covers a gel, if not, add more AmBic.
15. Clean new eppendorf tubes with 100 µL of EB and label.
16. Transfer extracts to new labeled tubes.
17. Add 50 µL of EB to gel cuts, incubate for 15 min and transfer peptide extracts to corresponding tubes. Repeat.
18. Dry samples in speedvac to dry.
19. Resuspend a pellet in 10-15 µL of FA.
20. Measure peptide concentration using nanodrop. Ideal peptide concentration should be 500 ng -1 µg.
21. Clean the sample from salts/detergents using ZipTips.