

## Protein fractionation with 1D gel electrophoresis

### Protocol: 1D gel electrophoresis

SDS PAGE is the most commonly used low level protein separation and purification technique in biological research. The reason of using this technique in LC MS is to reduce sample complexity and to purify sample contaminants as detergents, salts, DNA and other non-protein compounds.

The procedures given below were optimised to enable the sensitive characterisation of proteins directly from one- dimensional polyacrylamide gels of varying thickness (0.5 – 1.5 mm) and acrylamide concentration (7 – 18%). For best results, gels should be used no earlier than one hour after casting to reduce acrylamidation of cysteine residues during electrophoresis.

For detailed information on casting the gel and handling the gel cassette, please refer to the downloadable documents on this site:

- Mini Protean II Cell instruction manual
- Mini Protean 3 Cell instruction manual

### Recipe for the separating gel

Amount			Reagent
10% gel	12,5% gel	15% Gel	
4.43 ml	3.387 ml	2.23 ml	H <sub>2</sub> O
3.15 ml	3.15 ml	3.15 ml	4x separating buffer pH 8.8
4.2 ml	5.243 ml	6.3 ml	30% acryl/bisacryl amide
0.6 ml	0.6 ml	0.6 ml	Glycerol
0.123 ml	0.123 ml	0.123 ml	10% ammonium persulphate
10 µl	6 µl	6 µl	TEMED

### Recipe for the stacking gel

Amount	Reagent
2.2 ml	H <sub>2</sub> O
1.0 ml	4x separating buffer pH 8.8
0.533 ml	30% acryl/bisacryl amide
0.224 ml	Glycerol
40 µl	10% ammonium persulphate
4 µl	TEMED

### Protocol: Coloring of the gel

Coloring can be done with silver staining or colloidal coomassie. In case of silver staining, use the following protocol, for it is optimized to be compatible with the purification and mass spectrometry methods used. Colloidal coomassie can be performed according to standard protocols.

## **Protocol: Optimised protocol for Silver staining of polyacrylamide gels**

### *Reagents*

- Fixation solution (40 : 10 : 50 v/v/v ethanol : acetic acid : water)
- Washing solution (30% v/v ethanol in water)
- Sensitising solution (0.02 % sodium thiosulfate)
- 0.1 % AgNO<sub>3</sub>
- Developing solution (0.05 % formaldehyde in 2 % sodium carbonate)
- 1 % acetic acid

### *Procedure*

1. After the gel has been run, fix the protein by incubating the gel slab in fixation solution for 20 - 30 minutes.
2. Incubate the gel in washing solution on a shaking platform for two times 20 minutes. Extended washing time helps to eliminate yellowish background usually observed after long developing of the gel.
3. Rinse the gel slab with water (2 changes, 1 - 2 minutes per change) and then leave it further in water for 20 minutes on a shaking platform.
4. Sensitise the gel with sensitising solution for 1 - 2 minutes. Discard solution and quickly rinse the gel slab with three changes of water (10 seconds each).
5. Incubate the gel in chilled 0.1 % AgNO<sub>3</sub> for 30 minutes at 4 °C (fridge).
6. Discard silver nitrate solution and quickly rinse the gel with two changes of water (30 seconds per each change).
7. Change the gel chamber.
8. Develop the gel with developing solution. Discard the developing solution as soon as it turns yellow and replace it with a fresh portion.
9. When a sufficient degree of staining has been obtained, quench staining by discarding the developing solution and replacement with 5 % acetic acid. Wash the gel with 1 % acetic acid several times and store in the same solution.
10. Wash the gel with milliQ water several times before cutting it to remove acetic acid

## **Protocol: fractionation of 1D gel**

### *Reagents*

- Scanner
- Real-size print of the gel
- Glass plate
- MilliQ
- Clean scalpel

### *Method*

1. Wear gloves in order to avoid keratin contaminations. Never touch the gel with bare hands.
2. Clean the scanner before use to avoid keratin contaminations. Make a scan of the gel. Print the scan in real size.
3. Plan the slices by drawing them on the real-size print. Code the slices. Prepare tubes for each slice.
4. Clean a glass plate to cut the gel on with soap and alcohol.
5. Place the gel on the glass plate and place the real-size print of the gel underneath.

6. Slice the lanes as planned. Put each slide in a separate tube. Keep the gel wet with milliQ to avoid breaking of the gel.
7. Prepare the slices for in-gel reduction, alkylation and digestion by chopping them in approximately 1x1 mm particles.

**For reduction, alkylation and trypsin digestion, see the protocol for in gel trypsin digestion on [www.proteomicsnijmegen.nl](http://www.proteomicsnijmegen.nl).**