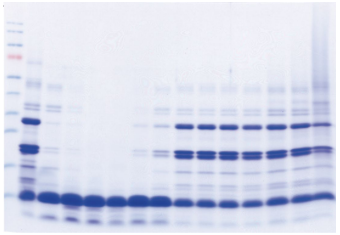
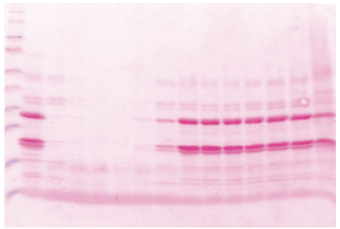
Laboratory 9

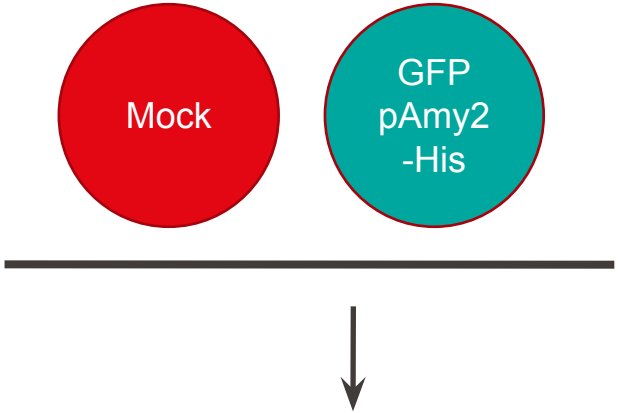
- SDS polyacrylamide gel electrophoresis (SDS-PAGE)
  - Coomassie blue staining



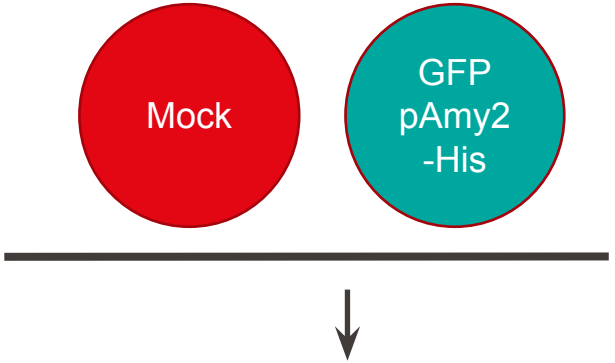
- Western Blot - Part I (transfer on nitrocellulose membrane)
  - Ponceau S Staining



Analysis of recombinant AMY2 expression upon transient transfection of HEK 293T cells (Lab9-11)



- **Transfection efficiency (Lab 7)**
  (number of GFP positive / total number of cells)

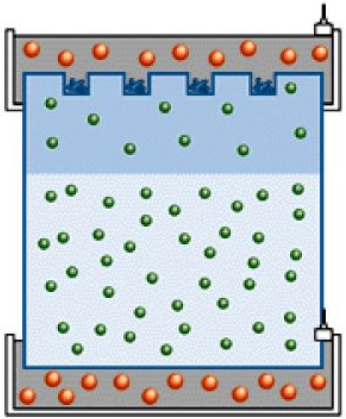


- **Cell lysates / Culture medium (Lab 8)**
- **Quantification / Affinity purification (Lab 8)**
- **SDS-PAGE (Lab 9)**
- **Western Blot (Lab 9 + 10)**
- Determine expression of AMY2-Myc-His fusion protein in transfected cells

SDS polyacrylamide gel electrophoresis (SDS-PAGE)

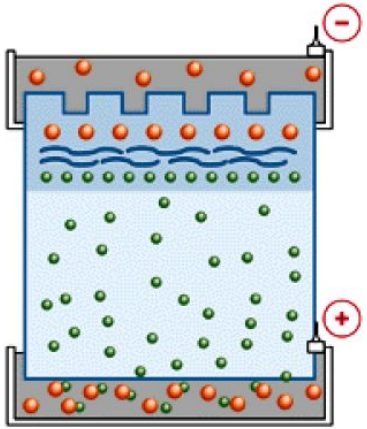
- Technique to separate proteins based on their **size**.
- In the presence of **sodium dodecyl sulfate (SDS)** proteins are linearized and **negatively charged**.
- Following separation, proteins are stained using **Coomassie-blue** (Gel 1)
- Proteins are transferred to a **nitrocellulose membrane** for western blot analysis (Gel 2).
- Proteins of interest are identified using **specific antibodies** (Lab 10).

SDS-PAGE buffer system



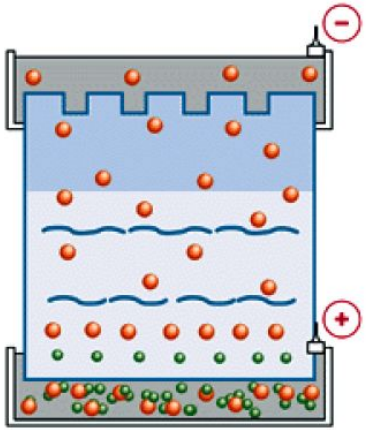
- Chloride ions (leading ions)
- Proteins

Electrophoreses buffer:
Tris-Glycine, pH 8.3



Stacking gel:
Proteins are trapped in a sharp band between ions.

Stacking gel buffer:
Tris-HCl, pH 6.8



Separation gel:
Proteins are separated according their size.

Separation gel buffer:
Tris-HCl pH 8.8

Adapted from: https://www.nationaldiagnostics.com/electrophoresis/article/multiphasic-buffer-systems

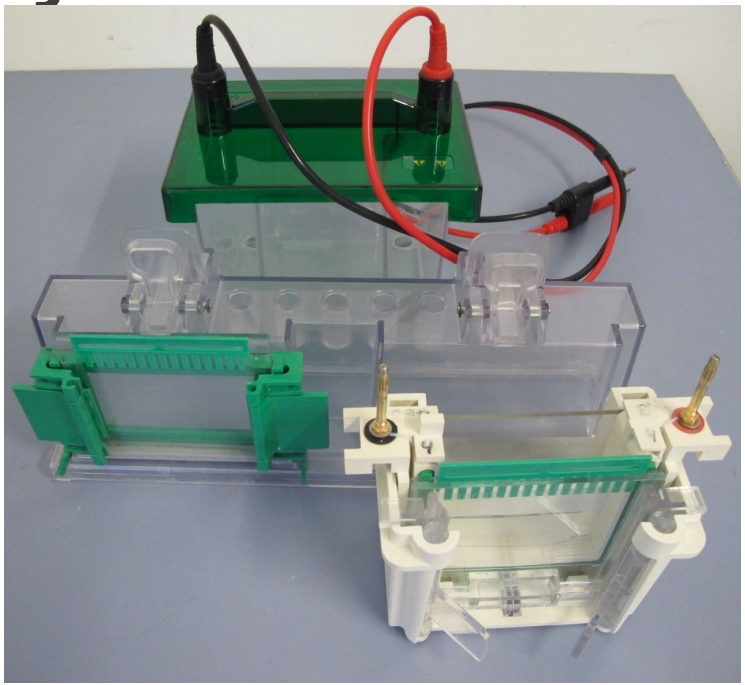
SDS-PAGE

- **Percentage of acrylamide**:
  - higher percentage give a better resolution for small proteins
  - lower percentage are preferred for larger proteins
  - gradients give optimal resolution for both small and large proteins

- **Sample loading buffer**
  - LDS (substitute for SDS) to denature proteins
  - glycerol for increased density during loading
  - blue + red dye for visulization of loading and migration

- **Prestained protein ladder** to visualize migration of proteins

Vertical SDS-PAGE system

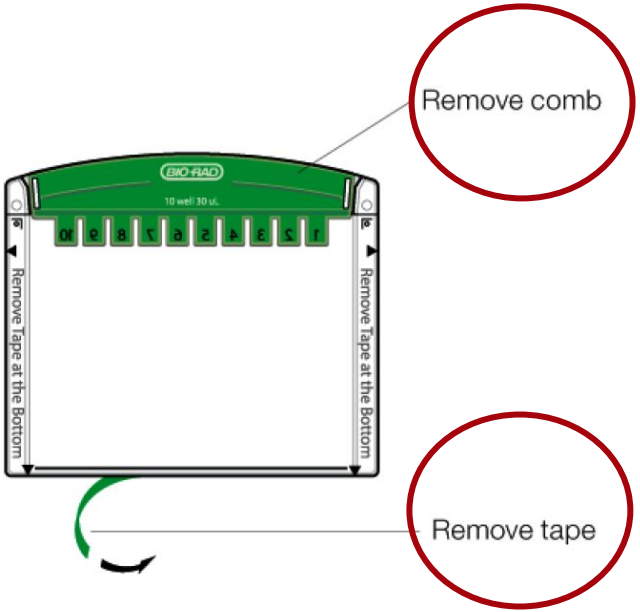


Electrophoresis tank

Gel casting stand
To save time we will use precast SDS gels

Gel chamber
For two gels, one on each side

Loading the samples on a precast SDS gel



Samples

1 Protein marker
2 L+
3 L-
4 M+
5 M-
6 TM+
7 FT
8 E1
9 E2
10 Porcine α-amylase

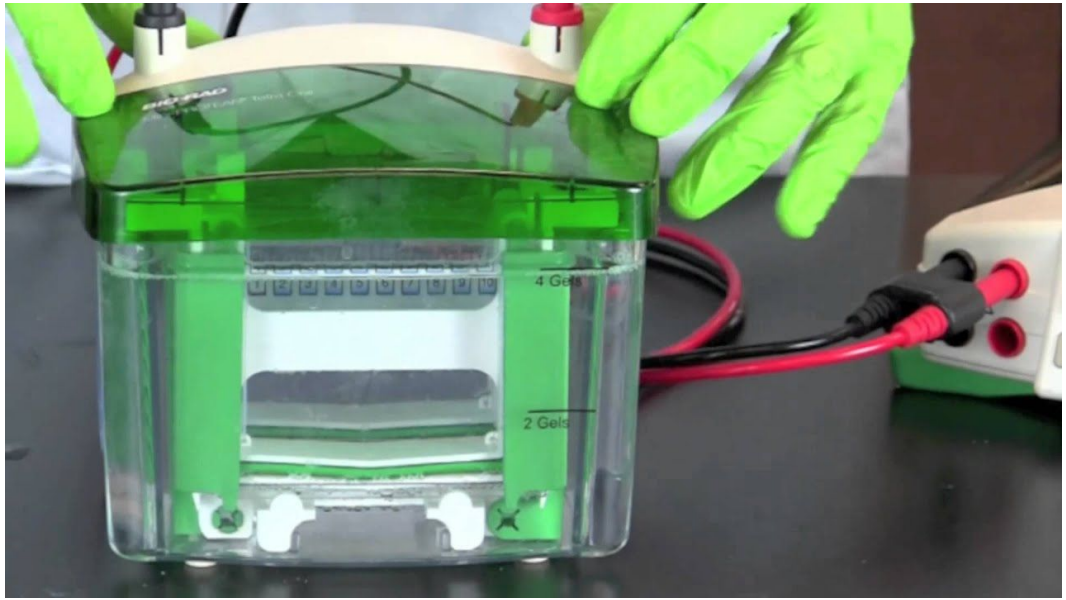
Gel 1: for Coomassie blue staining (Group X)

Gel 2: for Western blot (Group Y)

Gel loading

Put the gel (tape removed) into the gel running device, remove the comb and fill the tank with running buffer and carefully load the samples and ladder in the corresponding wells.

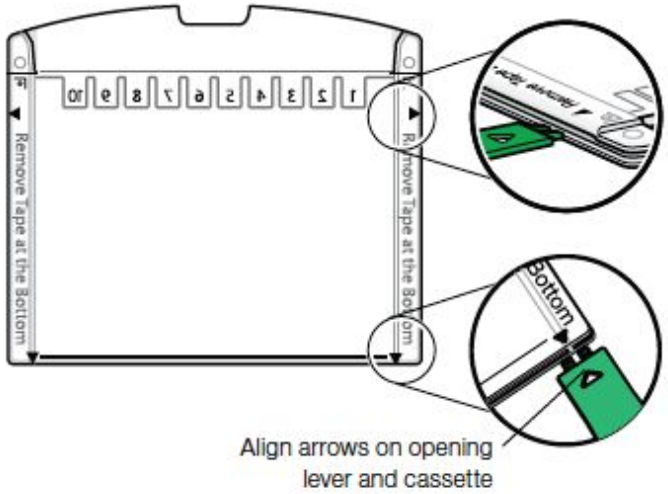
Gel running



After loading of samples, close the lid, switch on the current and migrate until the blue dye reaches the bottom of the gel

After migration of SDS-PAGE

Opening the Cassette



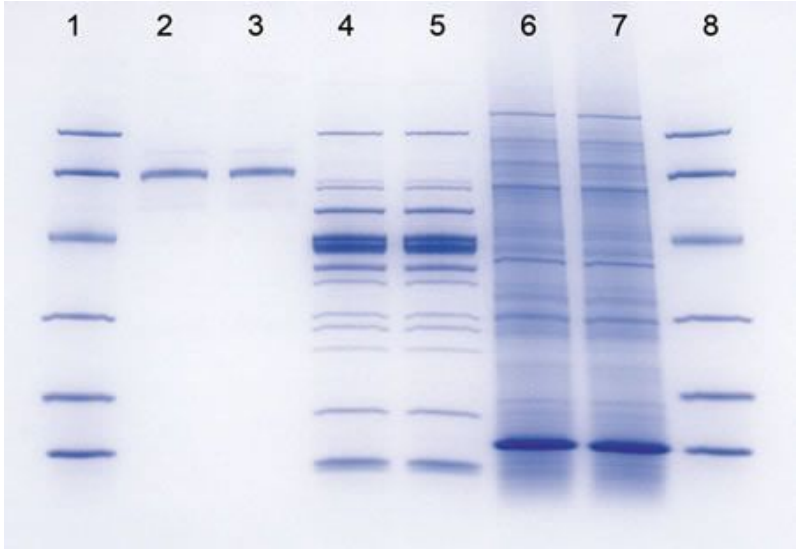
To open the cassette:

1. Align the arrows on the opening lever and on the cassette.
2. Insert the lever between the plates.
3. Apply downward pressure to break the seal.
4. Repeat the steps with the remaining three locations.

Staining and Tansfer of Proteins

- Gel 1: Coomassie-blue staining
- Gel 2: Transfer of proteins to nitrocellulose membrane for immunoblotting

Example of a Coomassie-stained gel

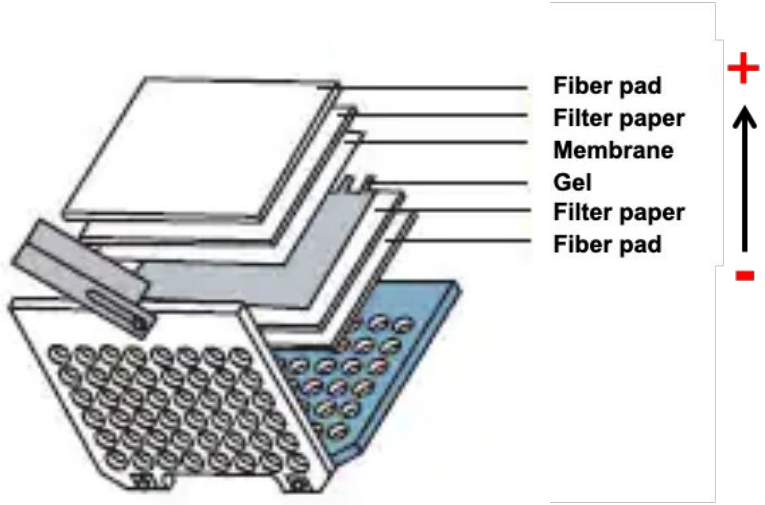


Protein stained with Bio-Safe Coomassie stain. Gel contains SDS-PAGE low range standards (lanes 1, 8), human transferrin (lanes 2, 3), fish lysate (lanes 4, 5), and *E. coli* lysate (lanes 6, 7).

http://www.bio-rad.com/en-ch/product/coomassie-stains?ID=2ef88af8-1ca6-44ef-9702-3ebcfe0ad35b

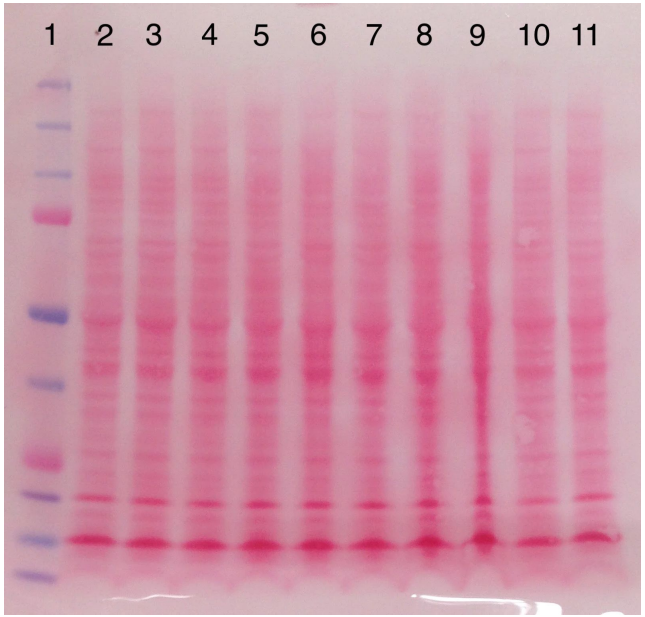
Equipment for Transfer of Proteins (Gel 2)

Setting up the Sandwich for Transfer of Proteins (Gel 2)



The proteins present in the gel will be transferred (blotted) onto the nitrocellulose membrane.
Always wear gloves when handling membranes to prevent contamination !

Example of a Ponceau S-stained nitrocellulose membrane



Rapid and reversible protein stain to check transfer.

Nitrocellulose membranes are stored in PBS at 4°C until incubation with specific antibodies.

Bubbles cause poor transfer