# Electrophoretic mobility shift assay (EMSA)

## Material and equipment

| Description |
| --- |
| Plates |
| Spacers |
| Clamps |
| Saran wrap |
| Whatman paper (GE Healthcare) |
| SDS 10% |
| Poly-dI dC (Sigma Aldrich) |
| DNA P Labeled Probe |
| 100X BSA (Sigma Aldrich) |
| 5X Binding buffer |
| 10X TBE Buffer |
| 30% Acrylamide stock (19:1) |

| Description |
| --- |
| Coomassie Blue |
| TRIS |
| APS (10%) |
| TEMED |
| Glycerol |
| Bromophenol blue |

## Experimental procedure

To make a NATIVE PAGE, we need to take certain materials in consideration:

- **Coomassie Brilliant Blue**: Dissolve 2.5 mg of Coomassie Brilliant Blue in 0.45 mL methanol. Add 0.45 mL of deionized water. Add 0.1 mL of glacial acetic acid.
- **Acrylamide solution (30%):** Dissolve 30 g of acrylamide and 0.8 g of bis-acrylamide in deionized water to a final volume of 100 mL.
- **Separating gel buffer (4):** Dissolve 18.15 g of Tris in about 75 mL of deionized water. Adjust pH with HCl to pH 8.8, and add deionized water to a final volume of 100 mL.
- **Ammonium persulfate solution (10%):** Dissolve 0.1 g of ammonium persulfate in a final volume of 1 mL of deionized water.
- **Sample solution (2):** 0.187 M Tris–HCl (pH 6.8), 30% glycerol, and 80 µg/mL Bromophenol blue.
- **Electrophoresis buffer for NATIVE:** Dissolve 28.8 g of glycine and 6 g of Tris in deionized water to a final volume of 2 L.
- **Electrophoresis buffer for SDS-PAGE:** Dissolve 3 g of TRIS, 18.8 g of glycine, 10 mL of SDS 10% in deionized water to a final volume of 1 L.

To prepare a NATIVE PAGE, we need to follow this method:

1. Thoroughly clean and dry glass plates, suitable spacers, and comb. Assemble glass plates, spacers, and comb as described by the manufacturer.
2. Mix 3.3 mL of acrylamide solution (30%), 2.5 mL of separating gel buffer (4), and 4 mL of deionized water.
3. Degas the mixture to avoid air bubbles in the gel after polymerization and to remove oxygen in the gel solution which otherwise accelerates the polymerization process.
4. Add 100 µL of ammonium persulfate solution (10%) and 10 µL of TEMED which will start the polymerization process.

5. Pour the mixed solution between the glass plates and add the comb.

6. Polymerize the acrylamide for 15 minutes.

7. Remove the comb carefully. The gel is ready to use.

To prepare an SDS-PAGE follow the next concentrations:

| Stacking 5-6% | |
|---|---|
| Distilled water | 2.7 mL |
| Acrylamide (30%) | 670 uL |
| Tris 8.8 | 500 uL |
| SDS (10%) | 40 uL |
| APS (10%) | 40 uL |
| TEMED | 5 uL |

| Separating 12% | |
|---|---|
| Destilled water | 5.0 mL |
| Acrylamide (30%) | 6.0 mL |
| Tris 6.8 | 3.8 mL |
| SDS (10%) | 140 uL |
| APS (10%) | 140 uL |
| TEMED | 15 uL |

For polyacrylamide gel electrophoresis, mix 10 µL of protein solution with 10 µL sample solution (2) containing glycerol and the dye bromophenol blue.

The conditions that we need to take into account for the polyacrylamide gel electrophoresis are:

1. Fill the apparatus with gel electrophoresis buffer.
2. Start electrophoresis immediately. (For a gel of 1 mm thickness and 15 cm length, apply about 150 volts (constant voltage) which will result in about 20 mA of current.
3. Remove the gel from between the glass plates.
4. After gel electrophoresis, stain the gel with either Coomassie Brilliant Blue or with silver.
5. Destain the gel with deionized water.

To incubate the protein with the aptamer, we based our research on an article describing the ratio of the specific and unspecific signal of the experiment. HEPES (10 mM at pH 6.5) was chosen as the binding buffer for the incubation of the aptamer with the CRP. The time of incubation proposed is 1 h at different temperatures: 4°C, 25°C and 37°C.

## References


[1] C. Arndt et al., (2008). Native Polyacrylamide Gels. [PDF]. Retrieved from: https://drive.google.com/file/d/1k21QSebQl0XKD2EatVHizrisE-F7cqFz/view

[2] S. Centi et al., (2008). Development of an optical RNA-based aptasensor for C-reactive protein. [PDF]. Retrieved from: https://drive.google.com/file/d/1DQxUadTM6K8q63iH-Z363Vo2RmygqpGI/view