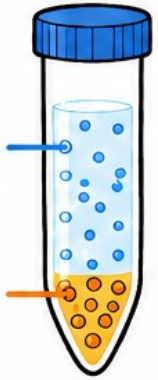
LAB SKILLS

1. Centrifugation

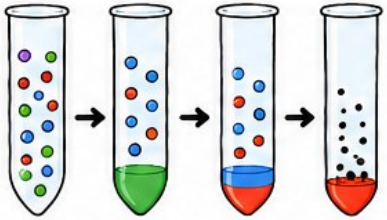
Separates substances of different densities.

SUPERNATANT — less dense, soluble and kept

PELLET — more dense, insoluble and removed



Successive increases in speed or time pellet increasingly less-dense substances.

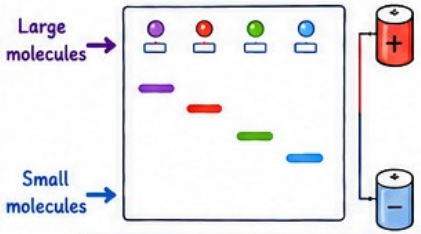


2. Gel Electrophoresis

Charged macromolecules move through a gel matrix in an electric field.

Native Gel Electrophoresis

Undenatured proteins are separated by size, shape and charge.

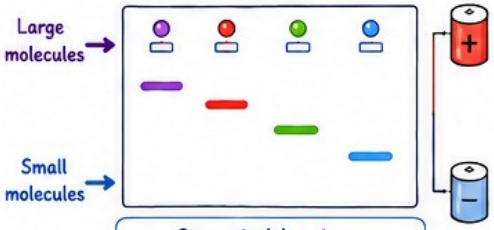


Separated by size, shape and charge

SDS-PAGE

Denatured proteins have a uniform negative charge and are separated mainly by size.

Smaller proteins move further.



Separated by size

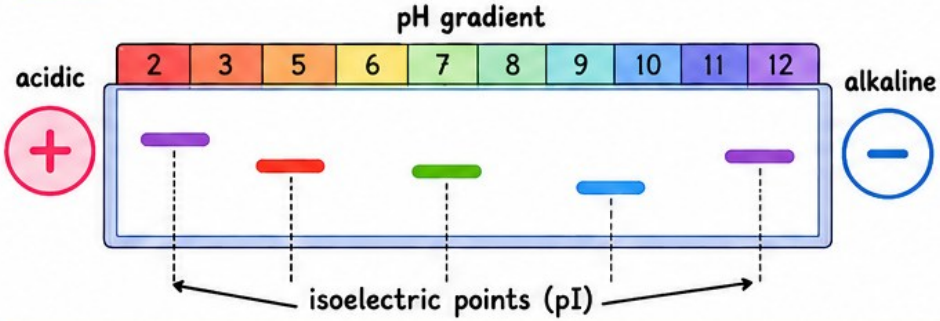
3. Isoelectric Focusing

Separates proteins by their different isoelectric points (pI).

Proteins move through a gel containing a pH gradient.

At pH = pI, the protein has no net charge and stops moving.

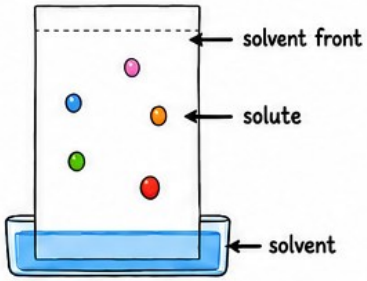
Buffers maintain a constant pH.



4. Chromatography

Paper / Thin-Layer

Separates substances according to their relative solubility in a solvent.



Affinity Chromatography

Target protein — high affinity; binds to beads.

Non-target proteins — low affinity; washed out.