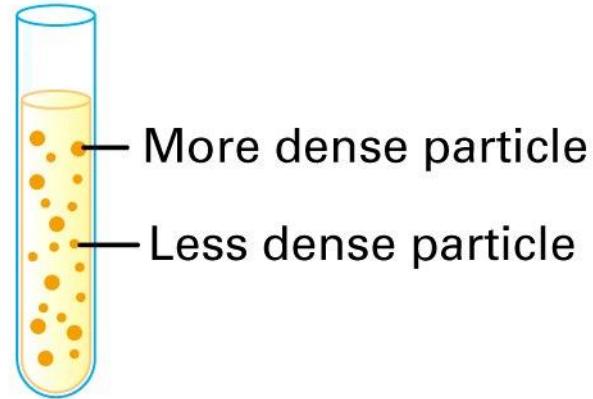


آزمایشگاه بیوشیمی

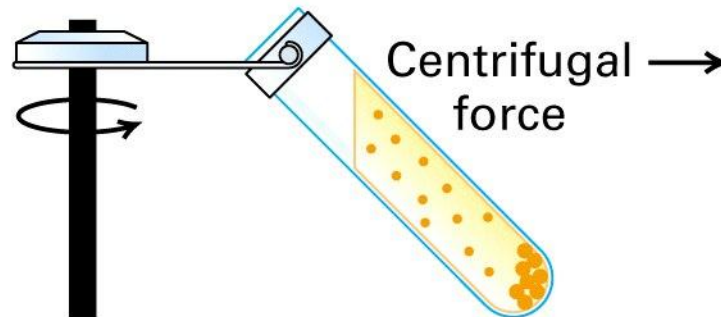
دکتر هادی انصاری هادی پور

(a) Differential centrifugation

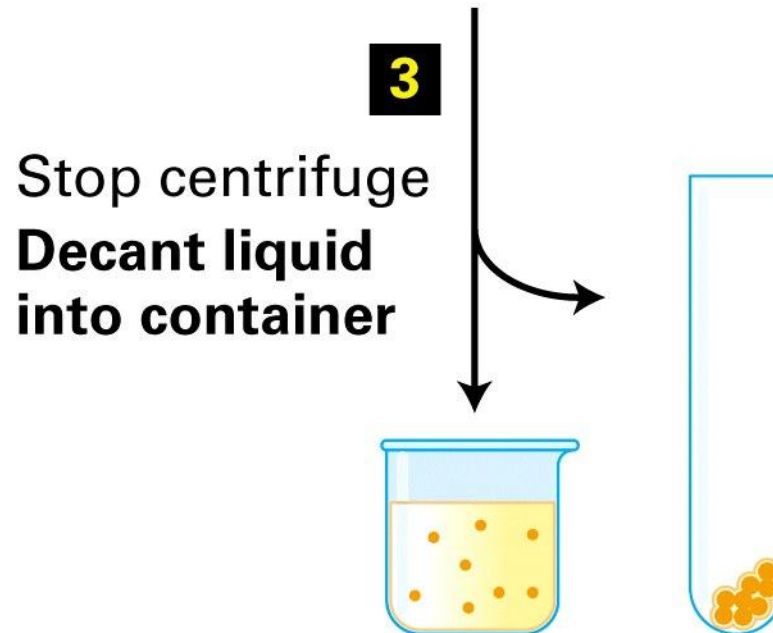
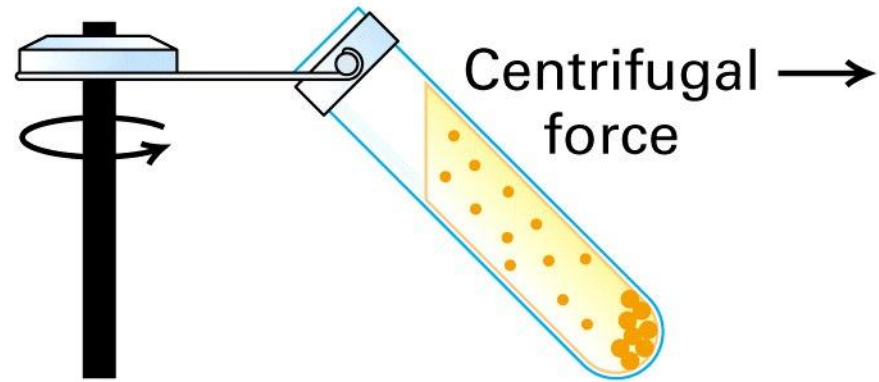
1 Sample is poured into tube



2 Centrifuge
Particles settle
according to
mass

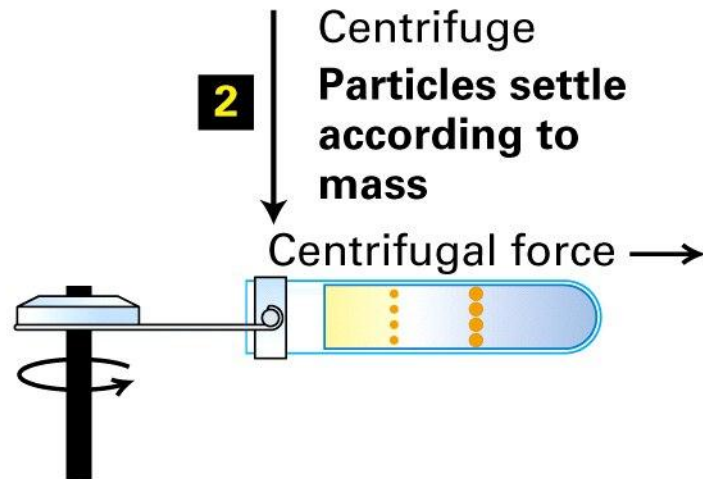
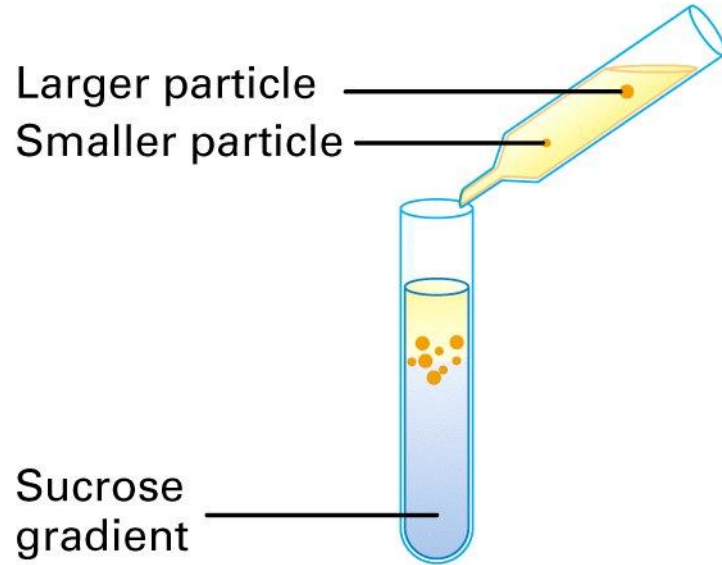


(a) Differential centrifugation

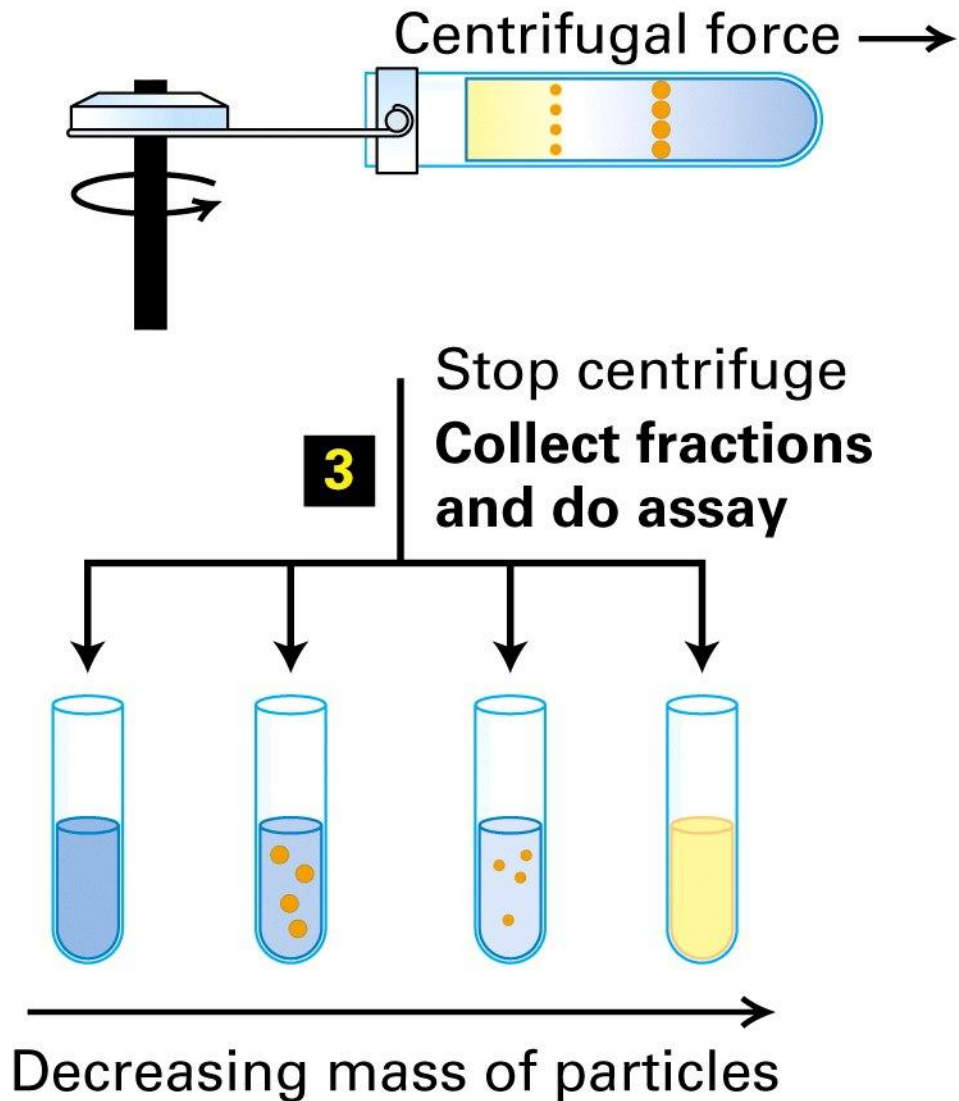


(b) Rate-zonal centrifugation

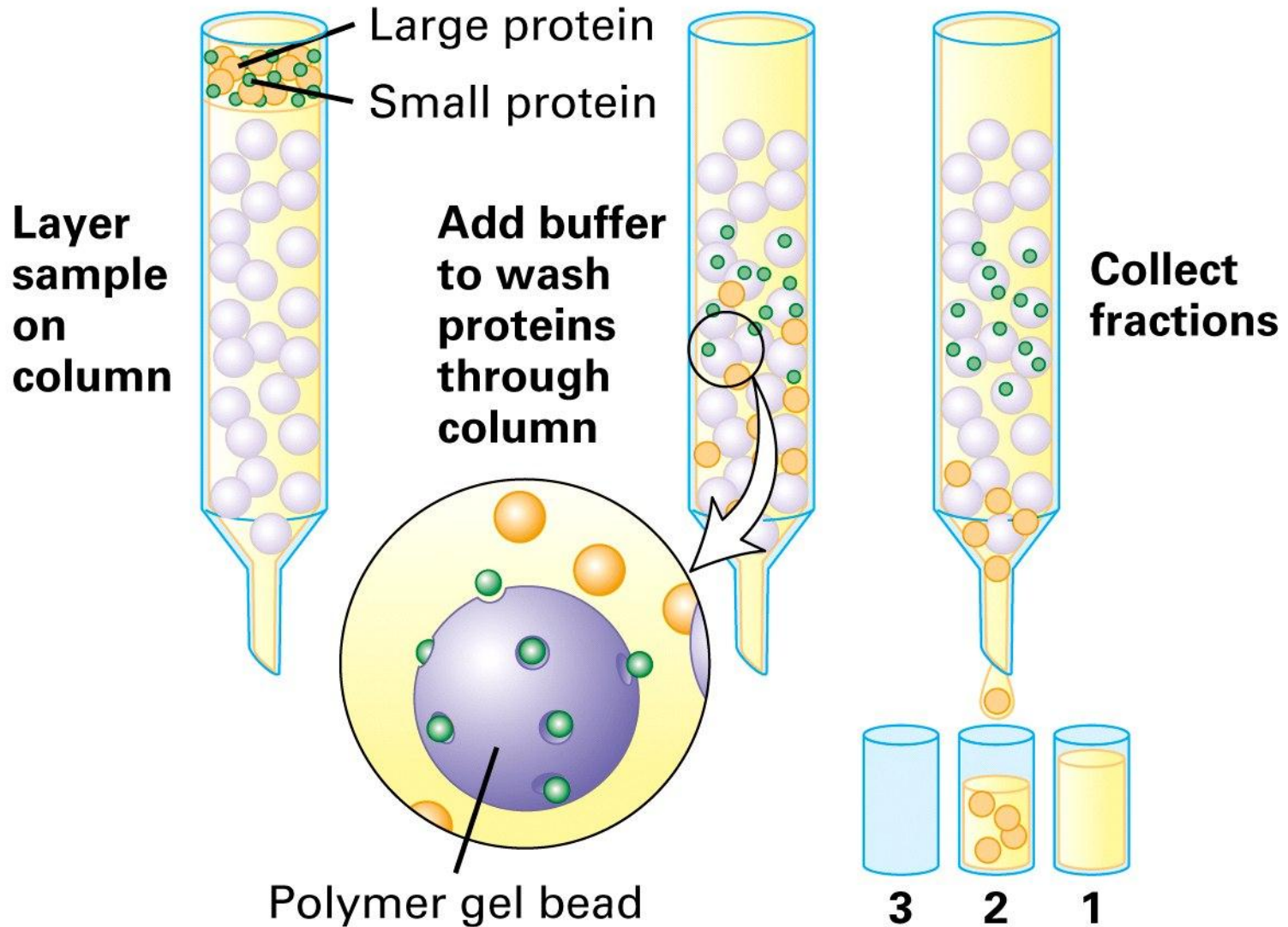
1 Sample is layered on top of gradient



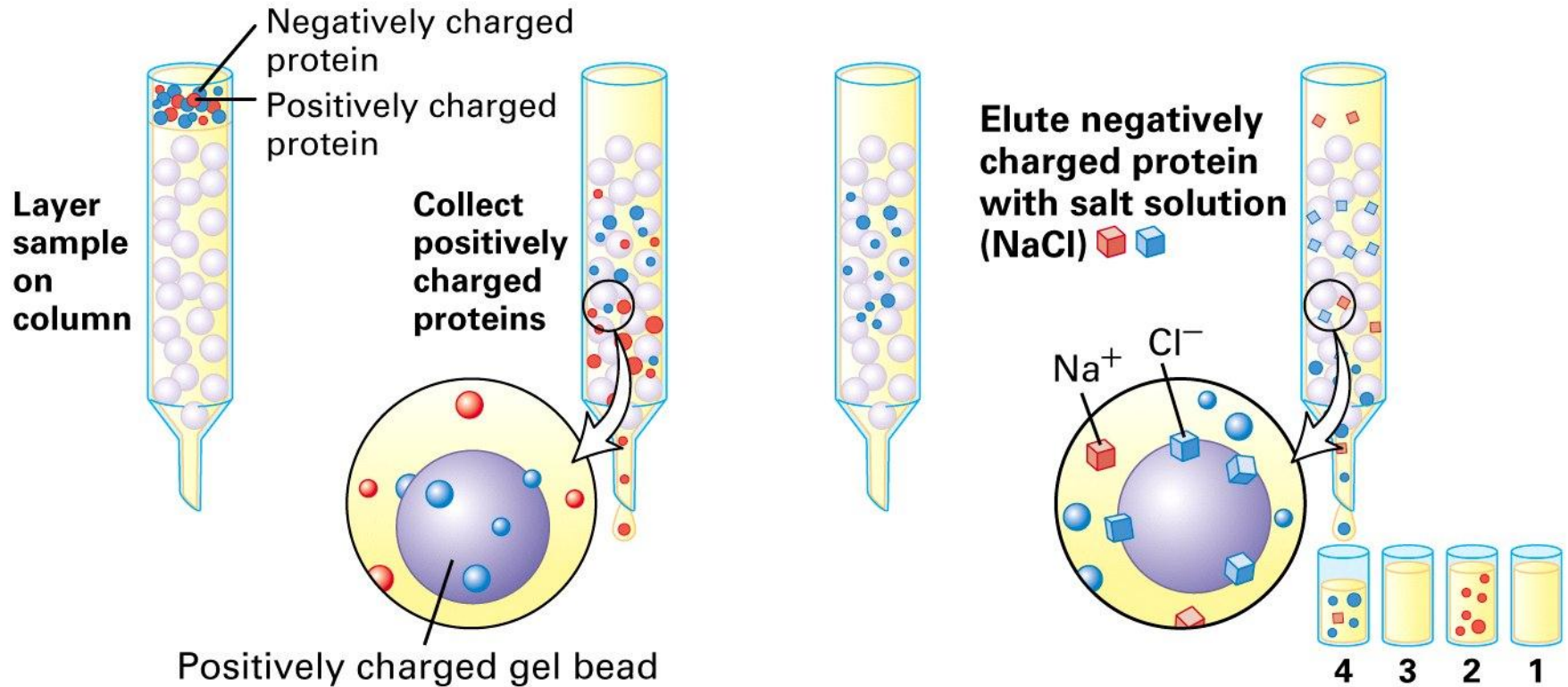
(b) Rate-zonal centrifugation



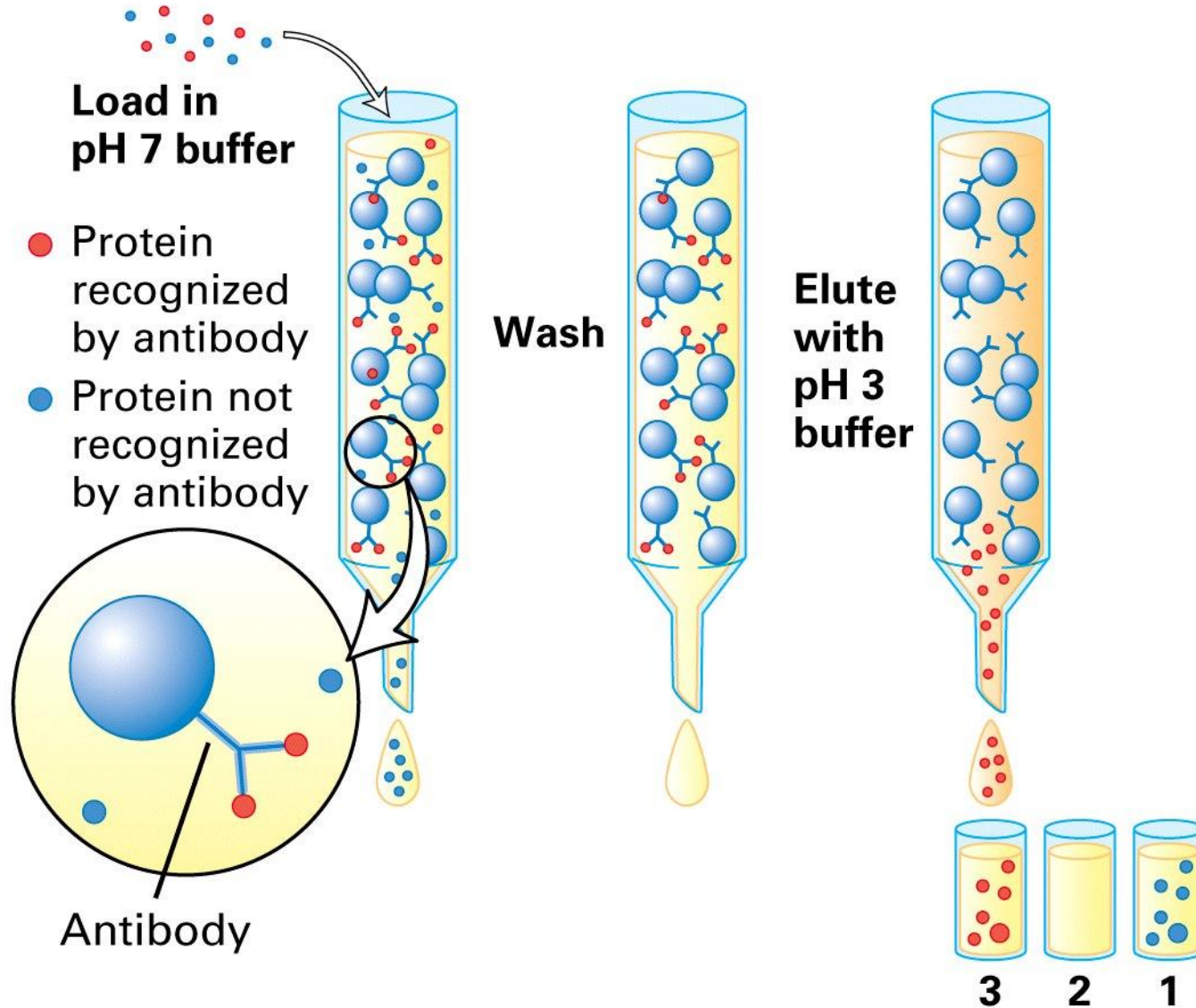
(a) Gel filtration chromatography



(b) Ion-exchange chromatography



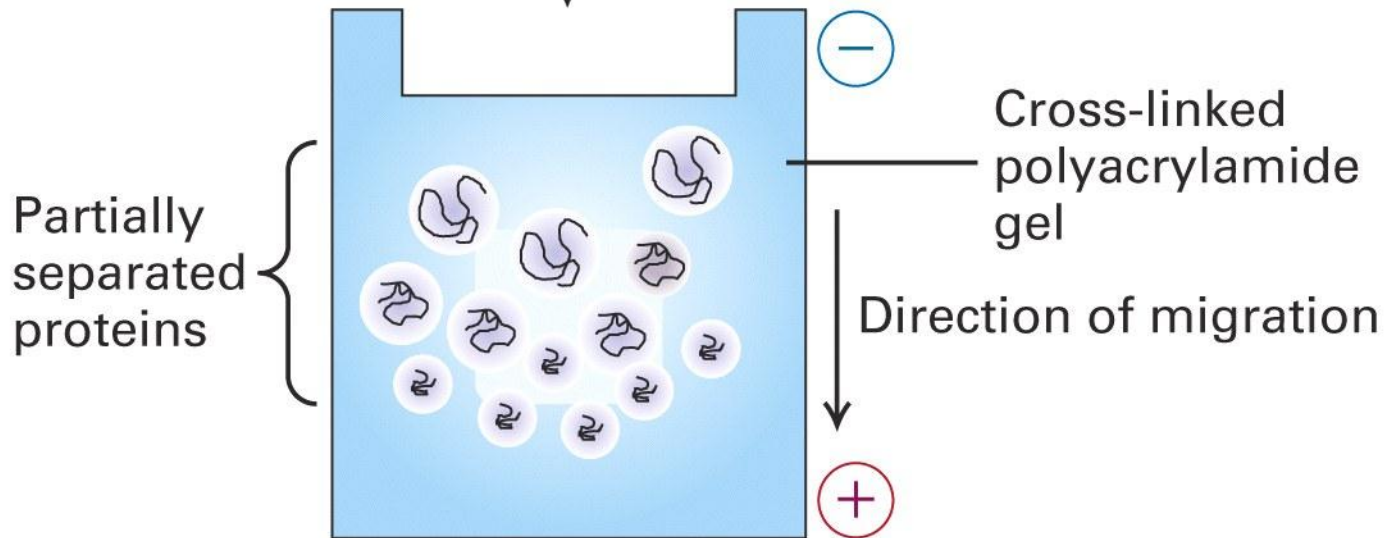
(c) Antibody-affinity chromatography

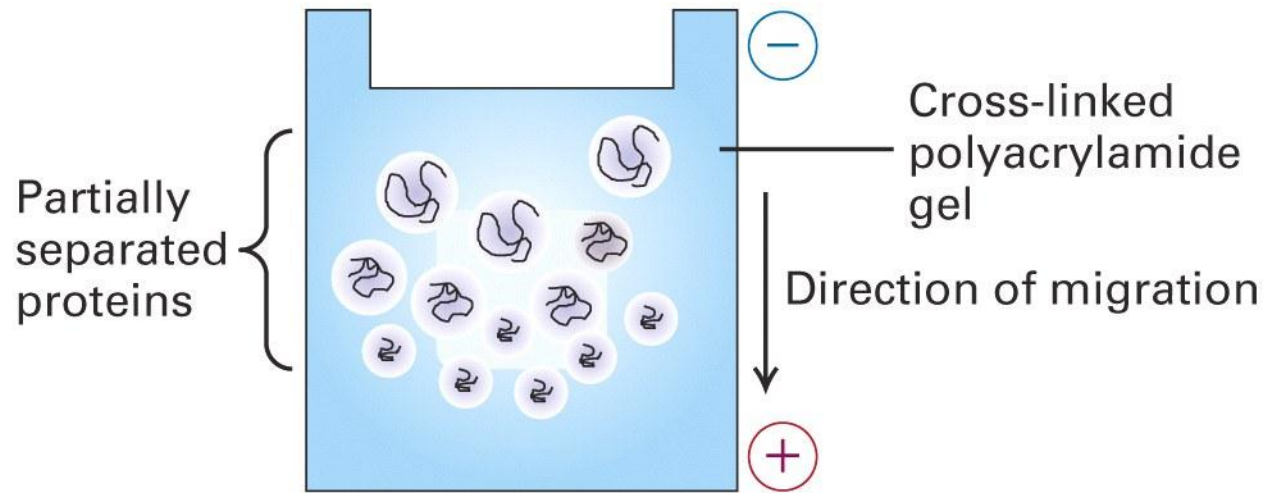


1 Denature sample with sodium dodecylsulfate

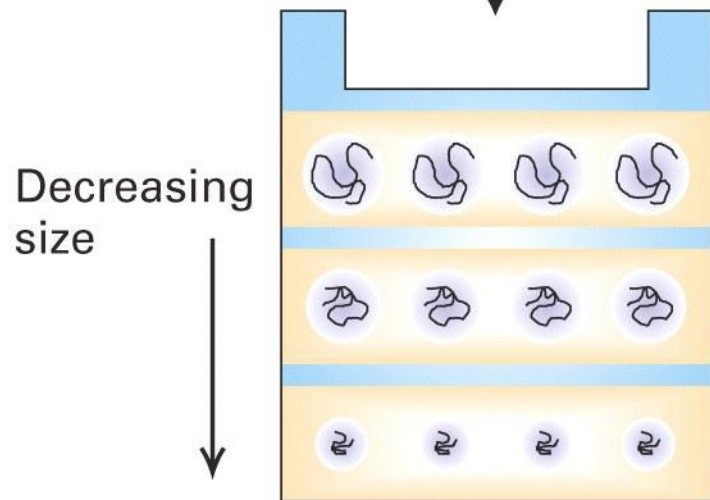


2 Place mixture of proteins on gel, apply electric field



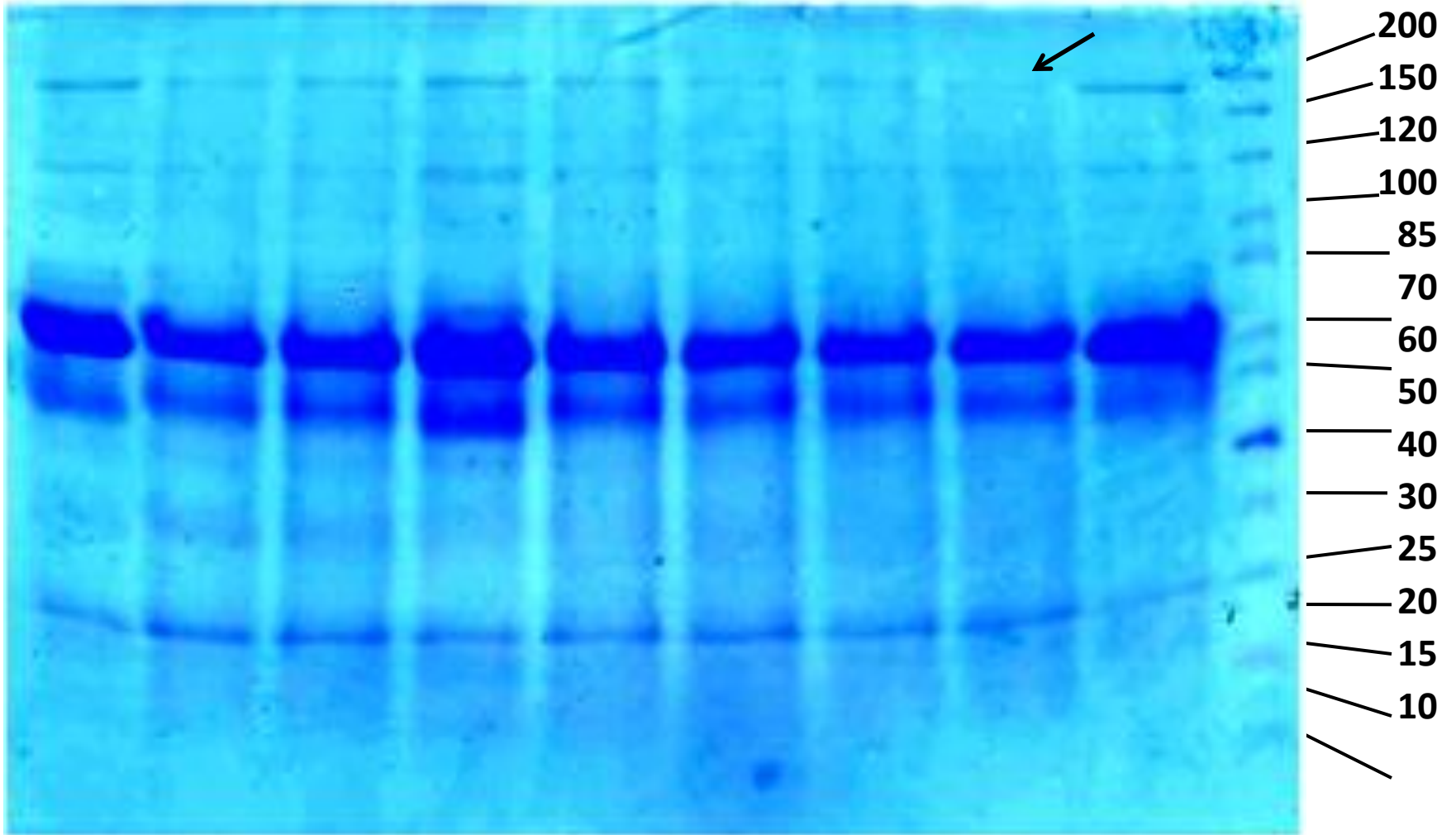


3 Stain to visualize separated bands

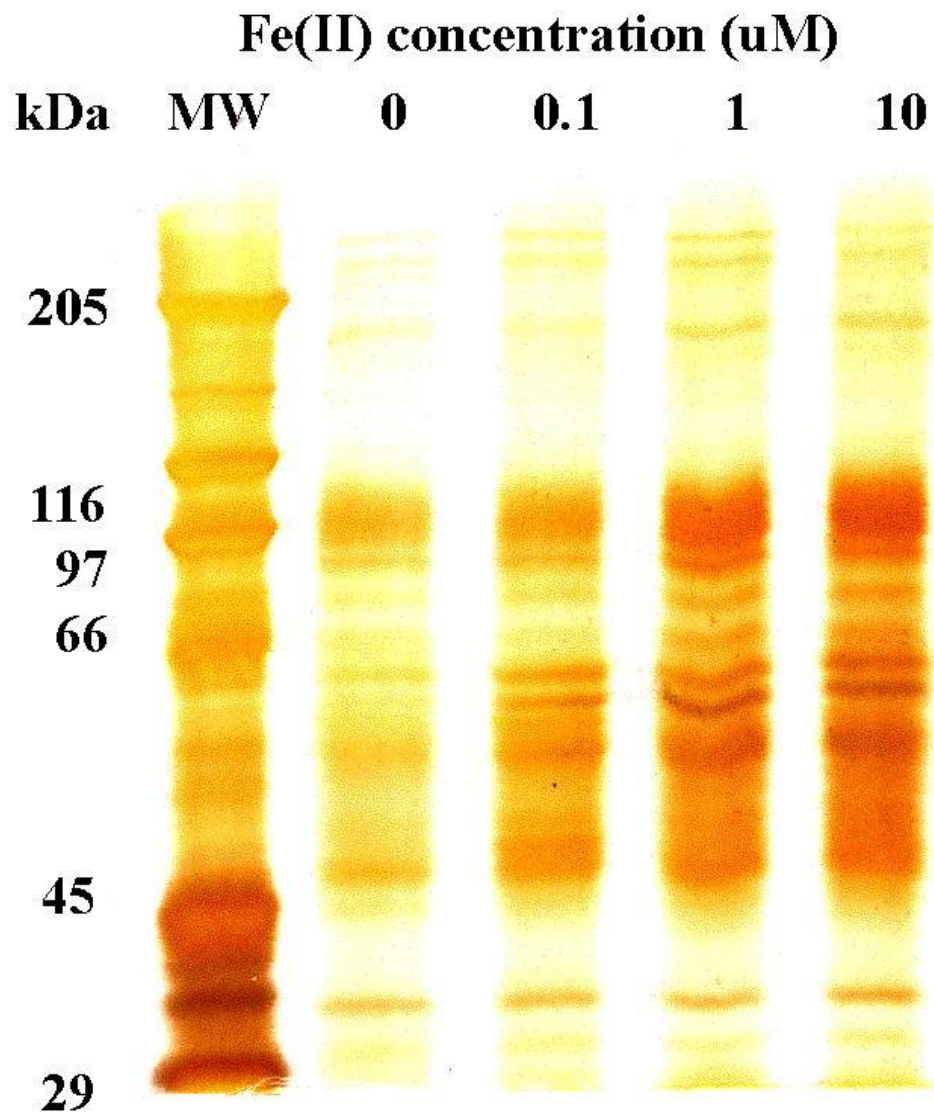


Fe⁺⁺ (mM)

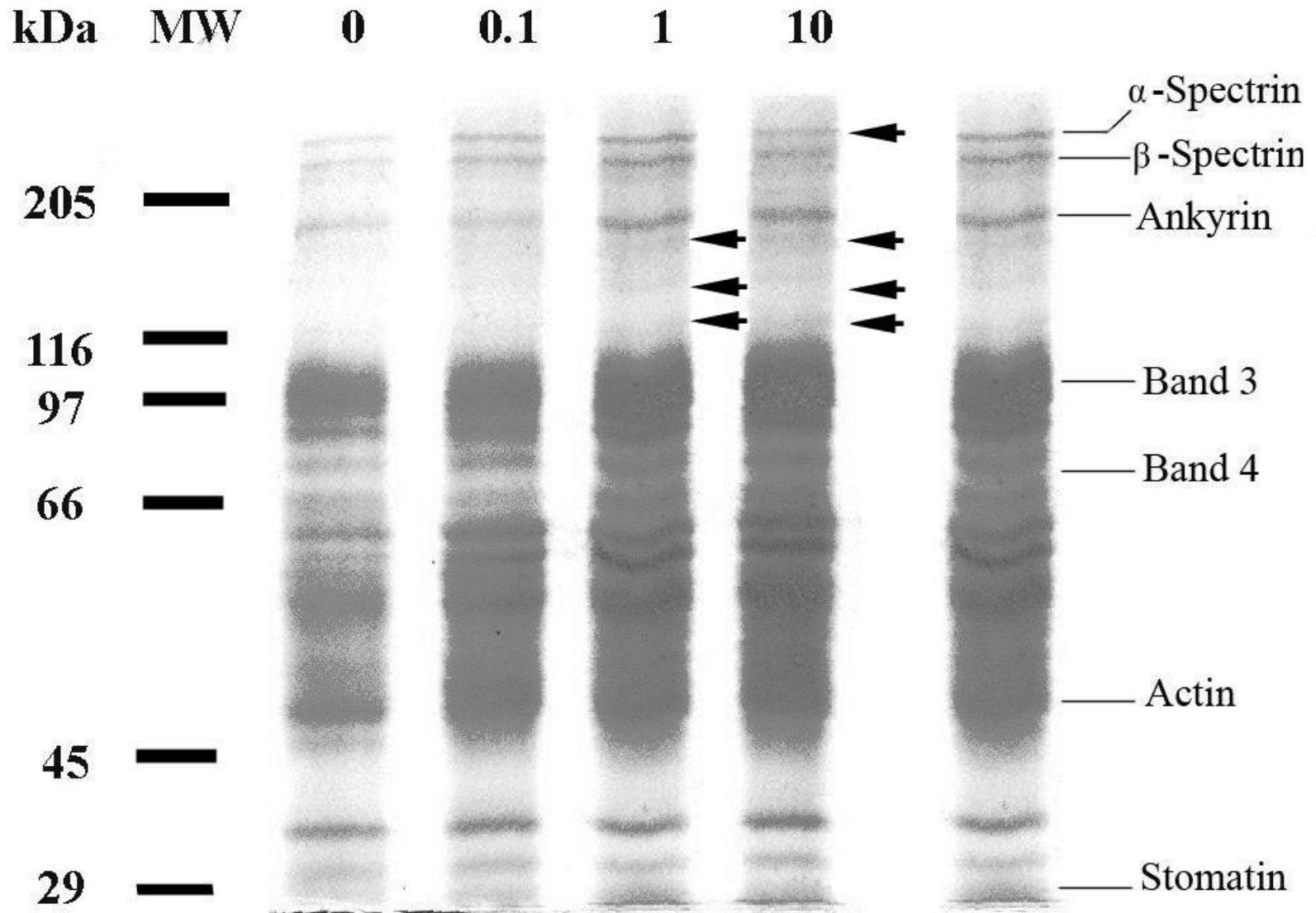
0 1.57 3.13 6.25 12.5 25 50 100 0 MW (kDa)

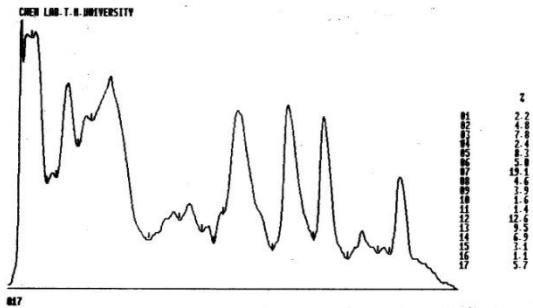


Coomassie BBG-250 stain

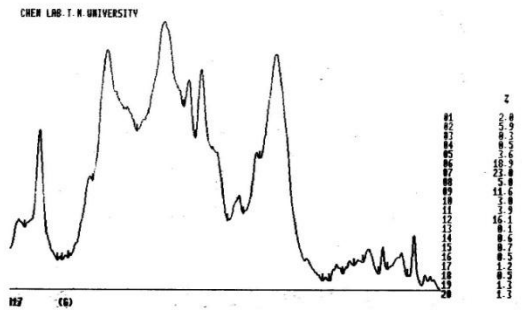


Iron Concentration (uM)

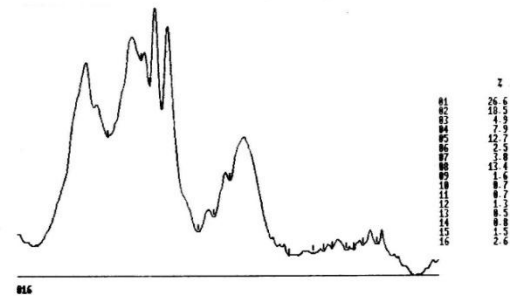




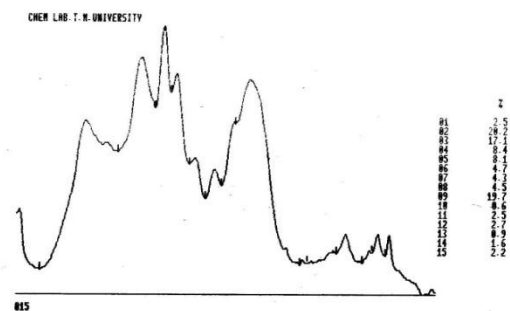
MW



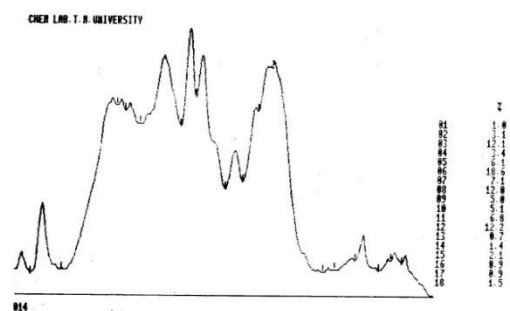
0 uM



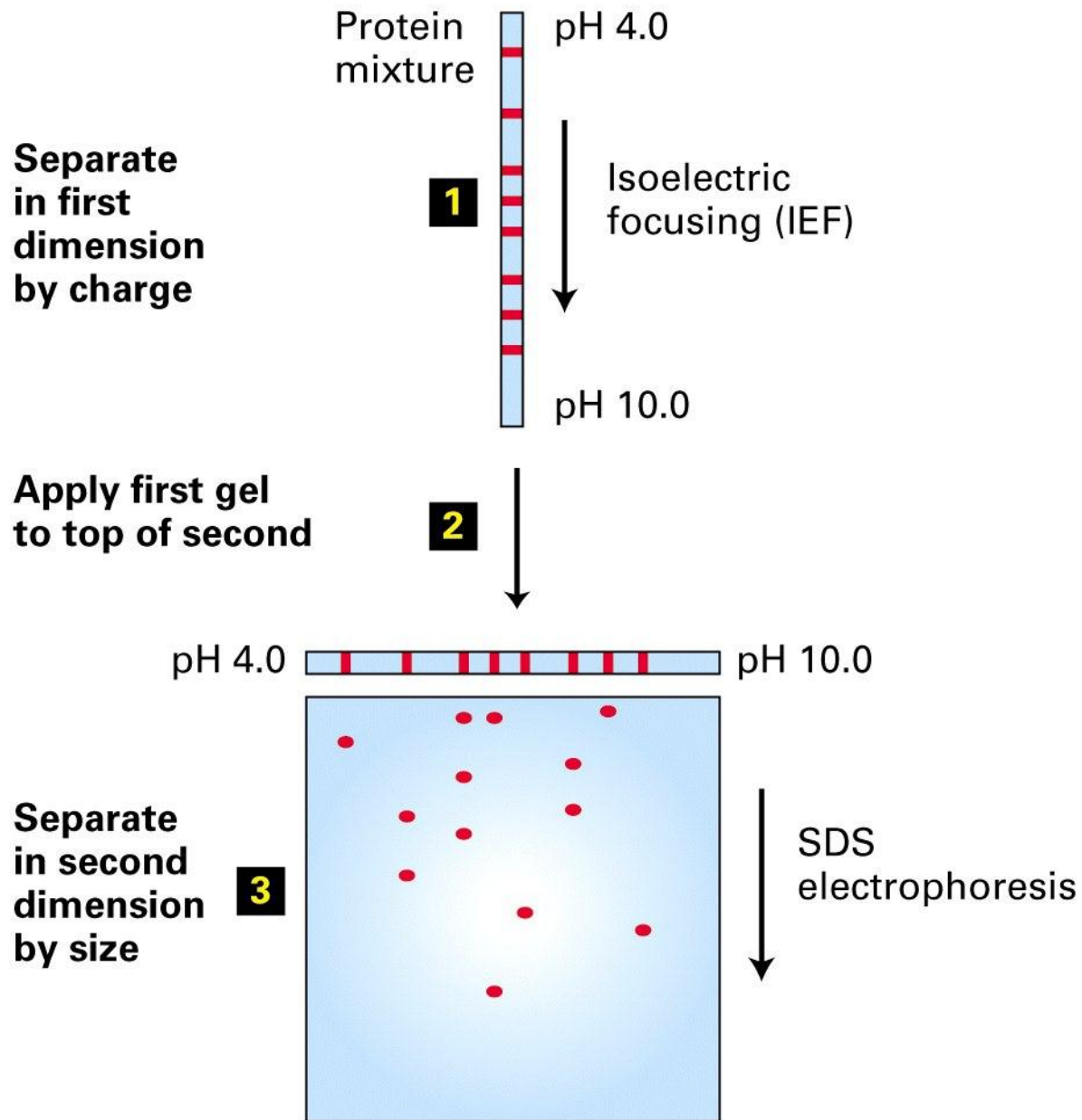
0.1 uM



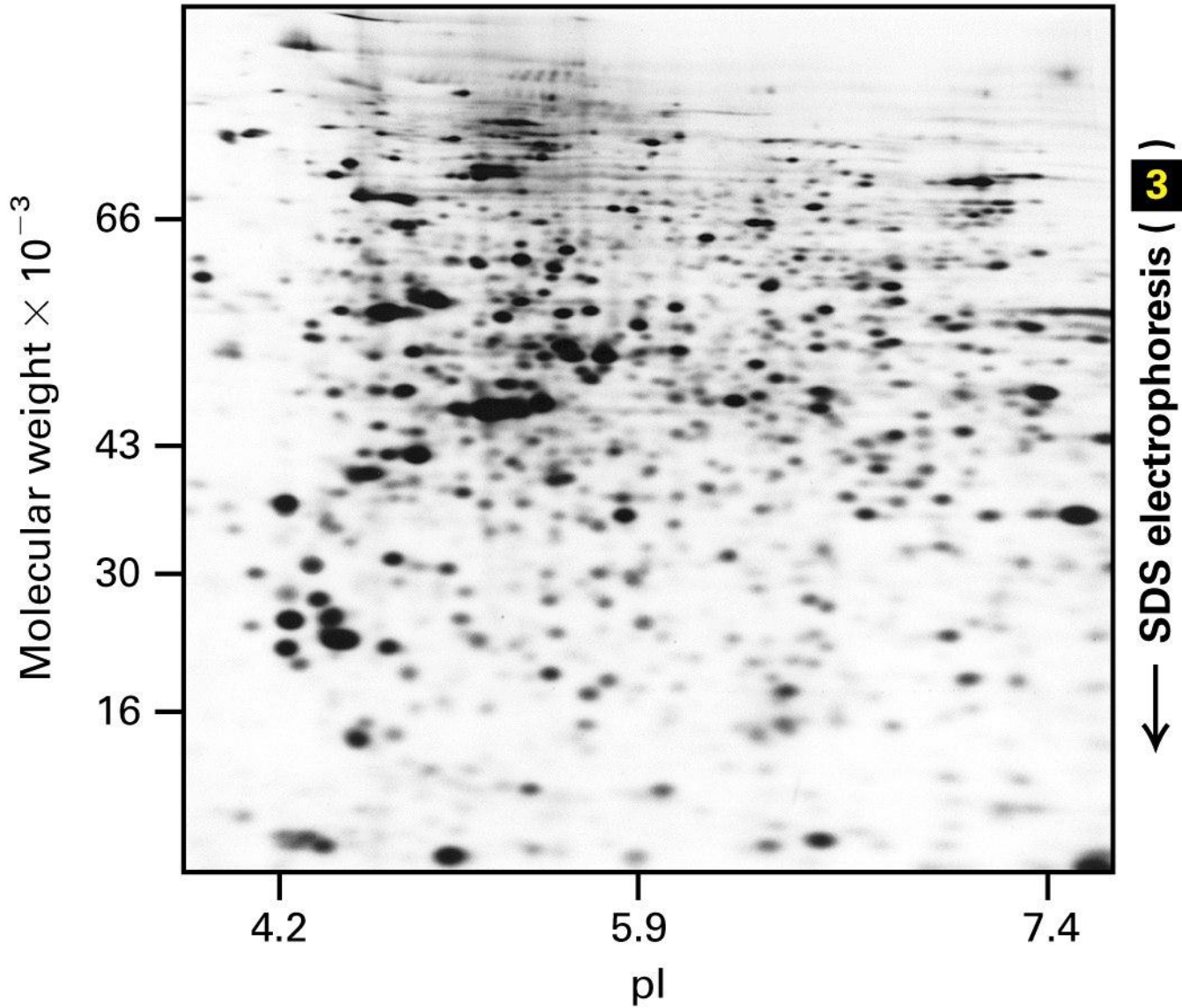
1 uM



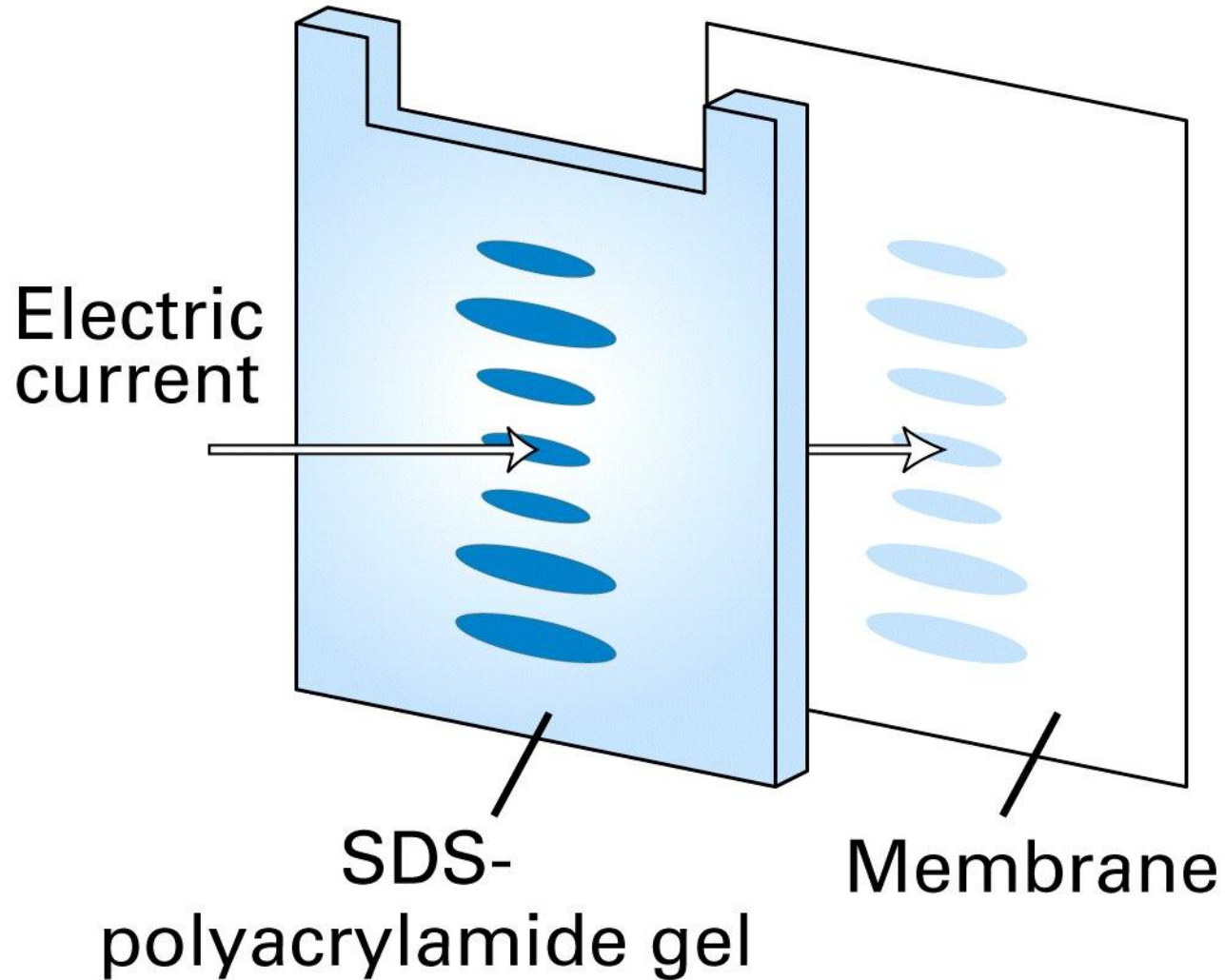
10 uM



Isoelectric focusing (**1**) →

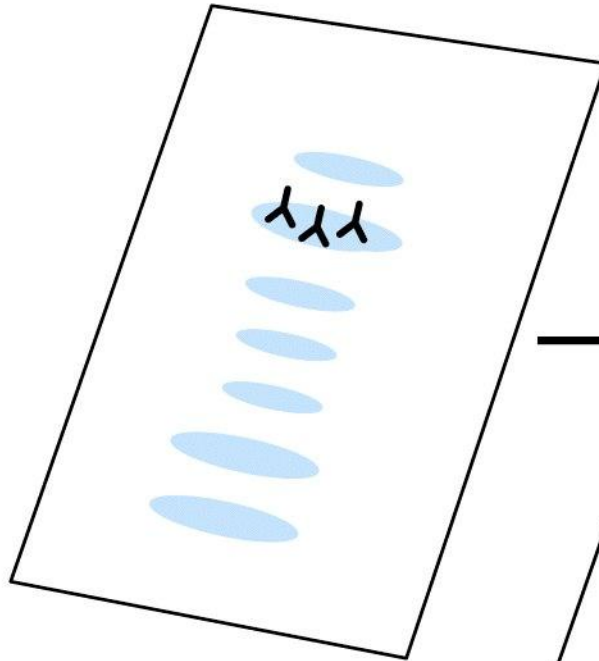


1 Electrophoresis/transfer



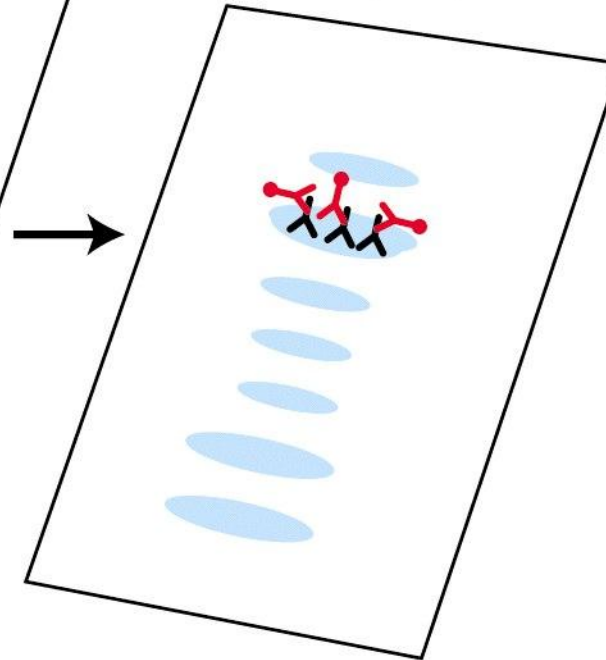
Antibody detection

2



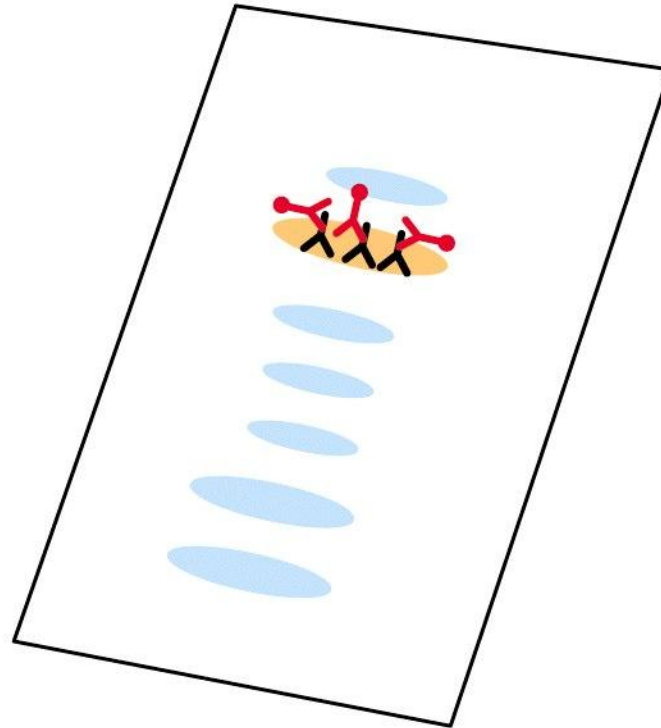
Incubate with
 Ab_1 (Y);
wash excess

3



Incubate with enzyme-
linked Ab_2 (Y);
wash excess

4 Chromogenic detection



React with substrate
for Ab₂-linked enzyme

TABLE 3-3**Radioisotopes Commonly Used
in Biological Research**

Isotope	Half-Life
Phosphorus-32	14.3 days
Iodine-125	60.4 days
Sulfur-35	87.5 days
Tritium (hydrogen-3)	12.4 years
Carbon-14	5730.4 years