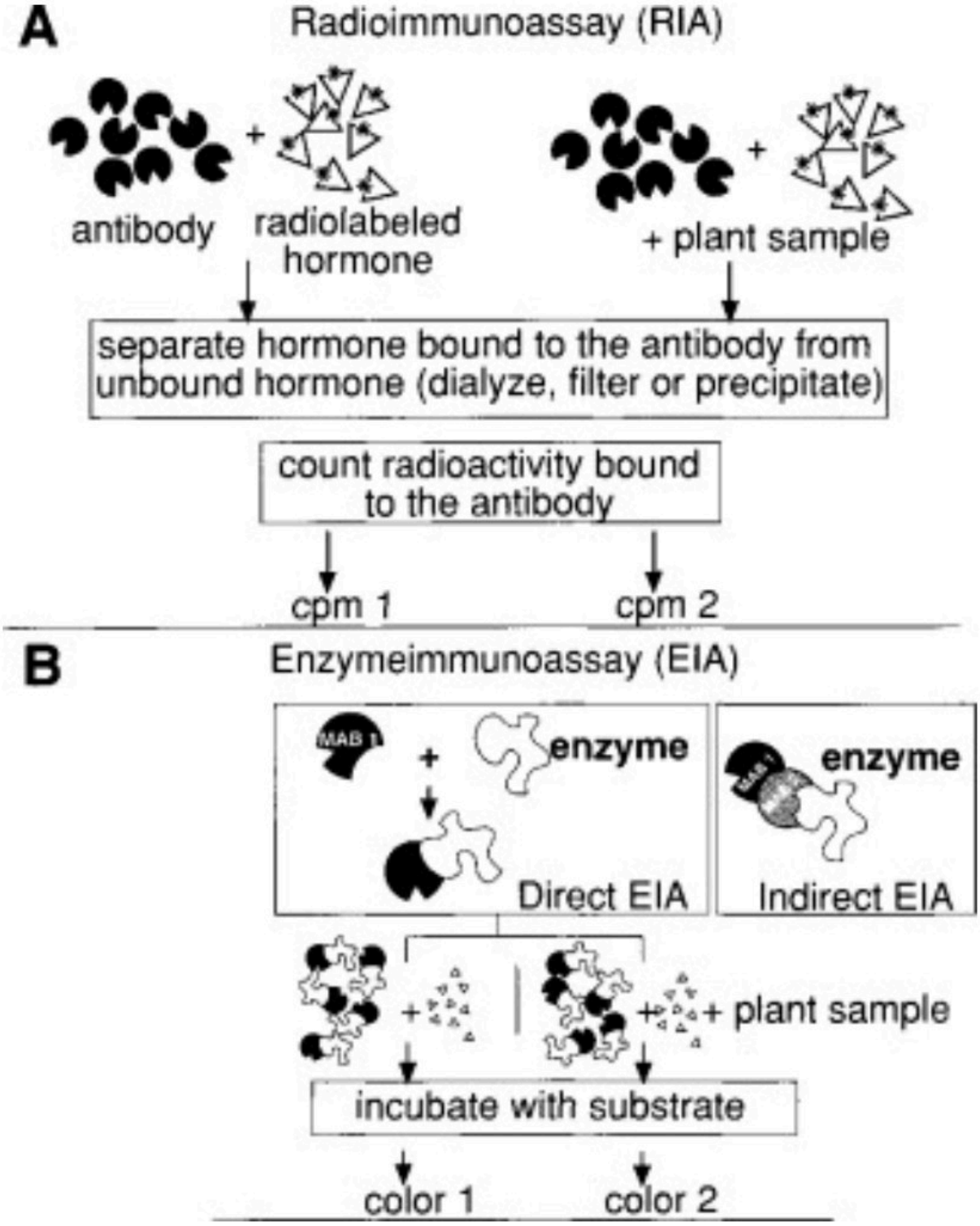
Radioimmunoassay requires a radioactively labeled hormone. A defined concentration of the radioactive hormone is incubated with a known quantity of antibody to which it binds, and then the free or unbound hormone is separated from that bound to the antibody by a suitable method (e.g., by dialysis or precipitation of the antibody by ammonium sulfate or by an antiantibody followed by filtration or centrifugation). The radioactivity bound to the antibody is counted in a scintillation counter (Fig. 5-6A). This is the control (cpm 1). Another mixture includes the same quantity of antibody and the same concentration of the radioactive hormone, plus the plant extract with the unknown amount of hormone. The radioactivity bound to the antibody in this mixture is also counted (cpm 2). The more unlabeled hormone in the plant extract, the less radioactivity is bound to the antibodies. Thus, the difference in radioactivity between the control vs the sample is proportional to the amount of the unlabeled hormone in the sample.


A
Radioimmunoassay (RIA)
antibody
radiolabeled hormone
+ plant sample
separate hormone bound to the antibody from unbound hormone (dialyze, filter or precipitate)
count radioactivity bound to the antibody
cpm 1
cpm 2
B
Enzymeimmunoassay (EIA)
MAB 1
enzyme
Direct EIA
enzyme
Indirect EIA
+ plant sample
incubate with substrate
color 1
color 2

The advantages of RIA are its relative simplicity and the high sensitivity provided by the use of radioactive compounds. However, there are several disadvantages as well: high specific activity-radiolabeled hormones and a scintillation counter are required, and they may not be easily available. Also, care is required in handling radioactive compounds.