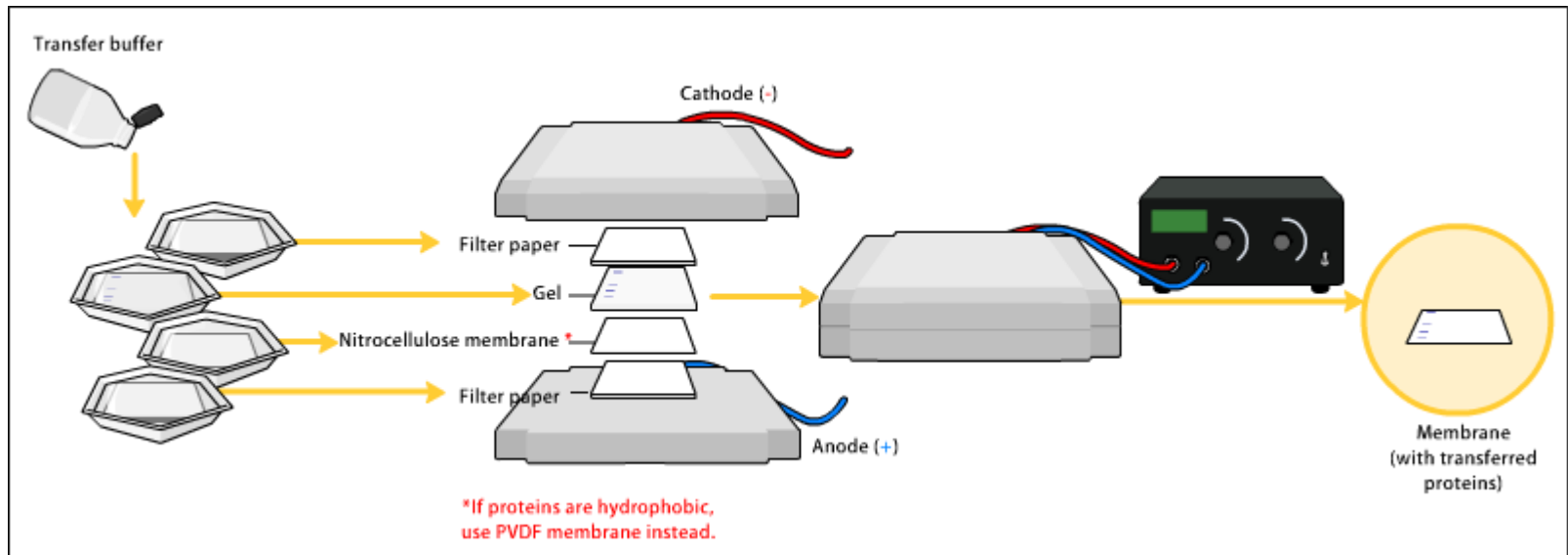


# Western Blotting



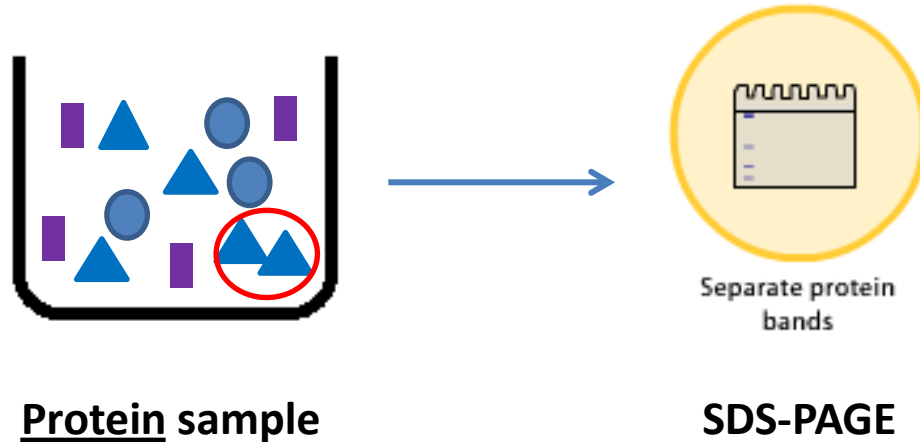
## Western blot:

- Is used to identify specific proteins, based on their ability to bind to antibodies.
- sometimes called the protein immunoblot

## Principle:

- 1- Proteins are separated from each other based on their size using SDS-PAGE.
- 2- Antibodies are used to detect the protein of interest.
- 3-A substrate that reacts with an enzyme is used to view the antibody/protein complex.

# Western Blot Steps



To confirm the separation of the sample use:

- 1-Replica of the gel and stain it as usual [with Coomassie brilliant blue] .
- 2-prestained marker.

## Western Blot Steps



## Western Blot Steps

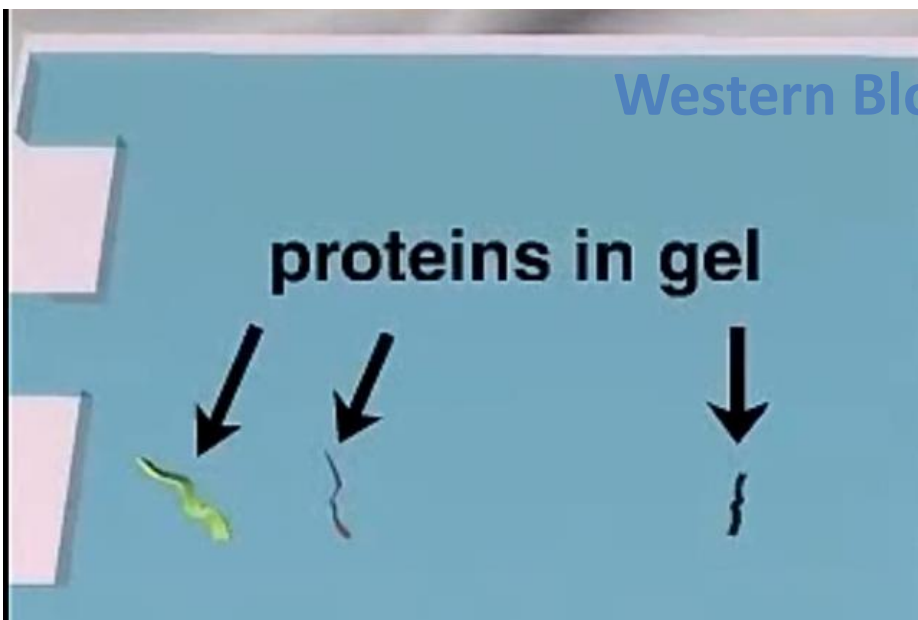
membrane sheet is placed on  
positive electrode



SDS-polyacrylamide gel



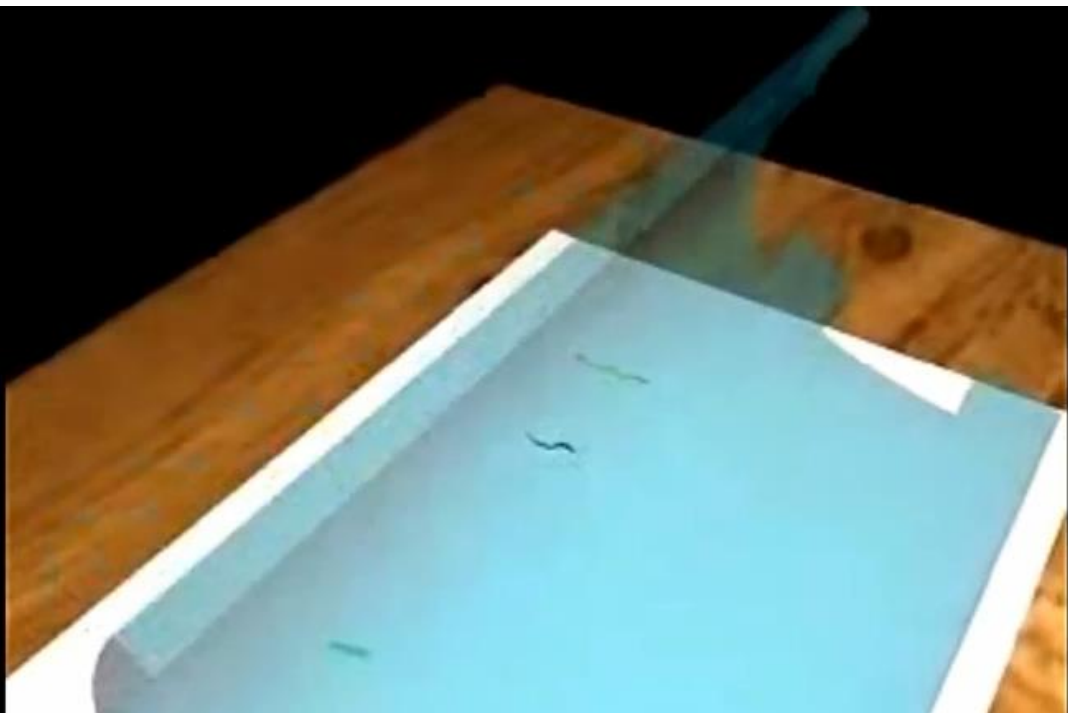
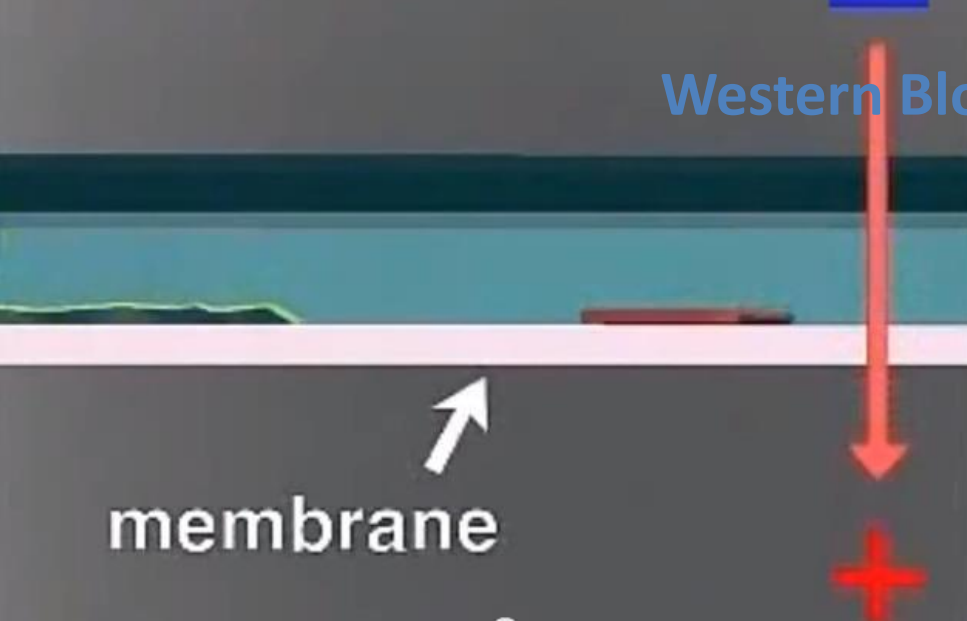
## Western Blot Steps



## Western Blot Steps

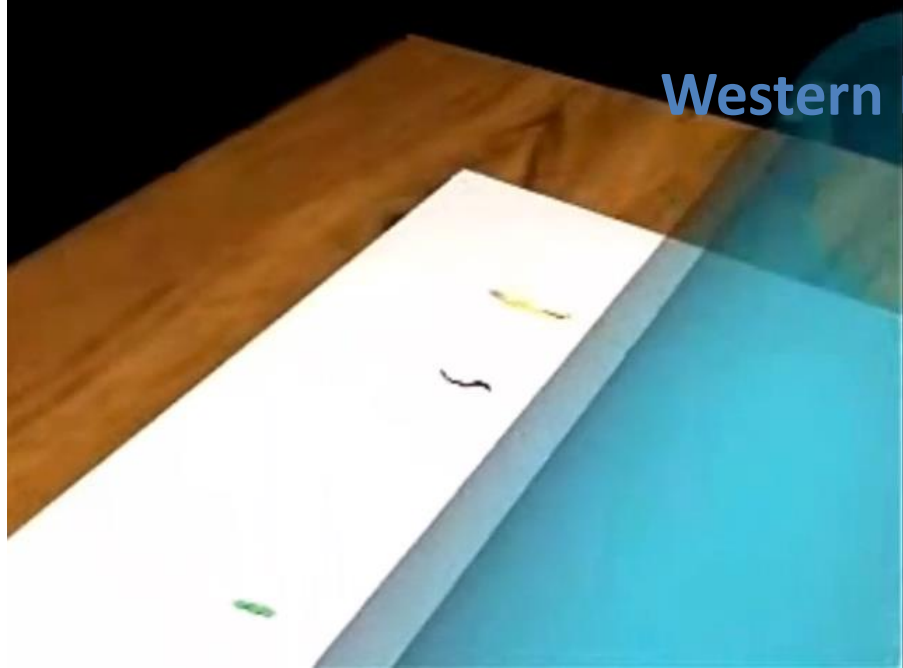


## Western Blot Steps





## Western Blot Steps



**primary antibodies (green)  
are added**

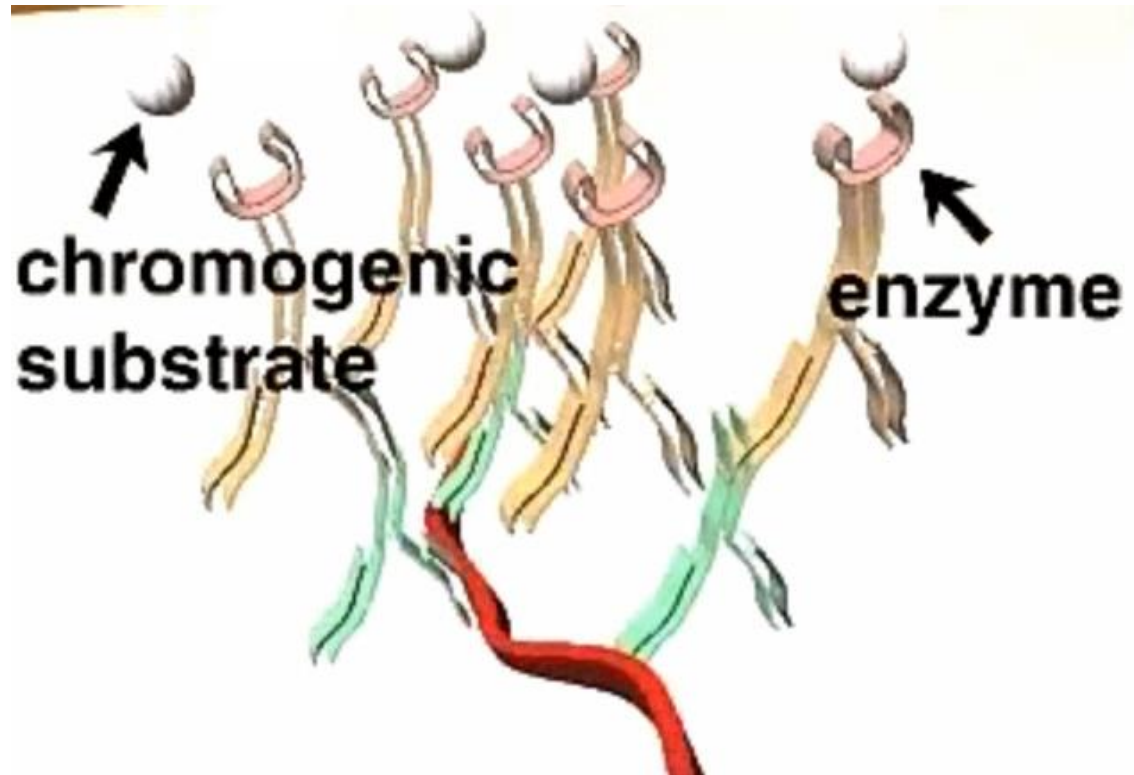
## Western Blot Steps



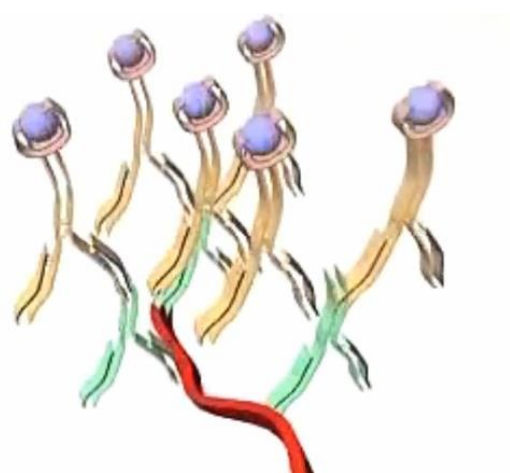
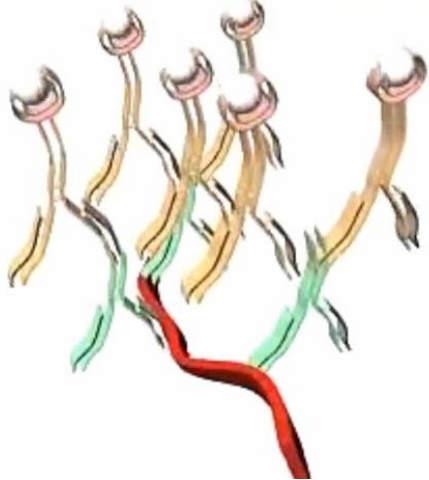
**enzyme-linked secondary  
antibodies (brown) are added**



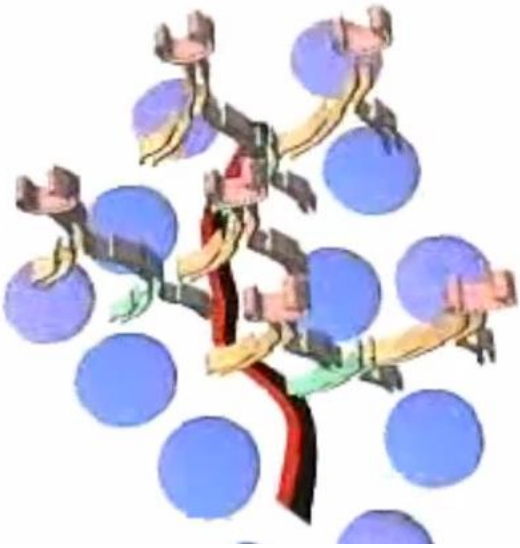
## Western Blot Steps



## Western Blot Steps



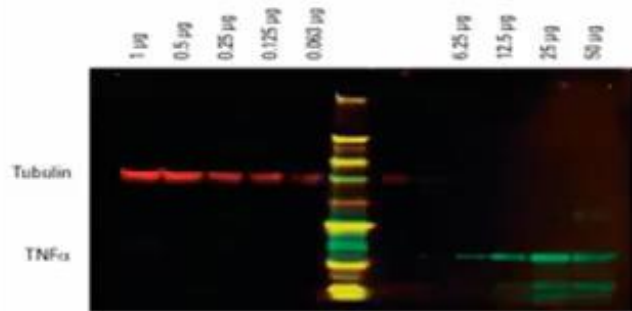
## Western Blot Steps



# Western Blot Steps



## COLOR WESTERN BLOT (FLUORESCENT DETECTION)



Imaged with the  
LI-COR Odyssey  
Infrared Imaging  
System.

DyLight 680/800 labeled  
antibodies



## Western Blot Steps Summary

