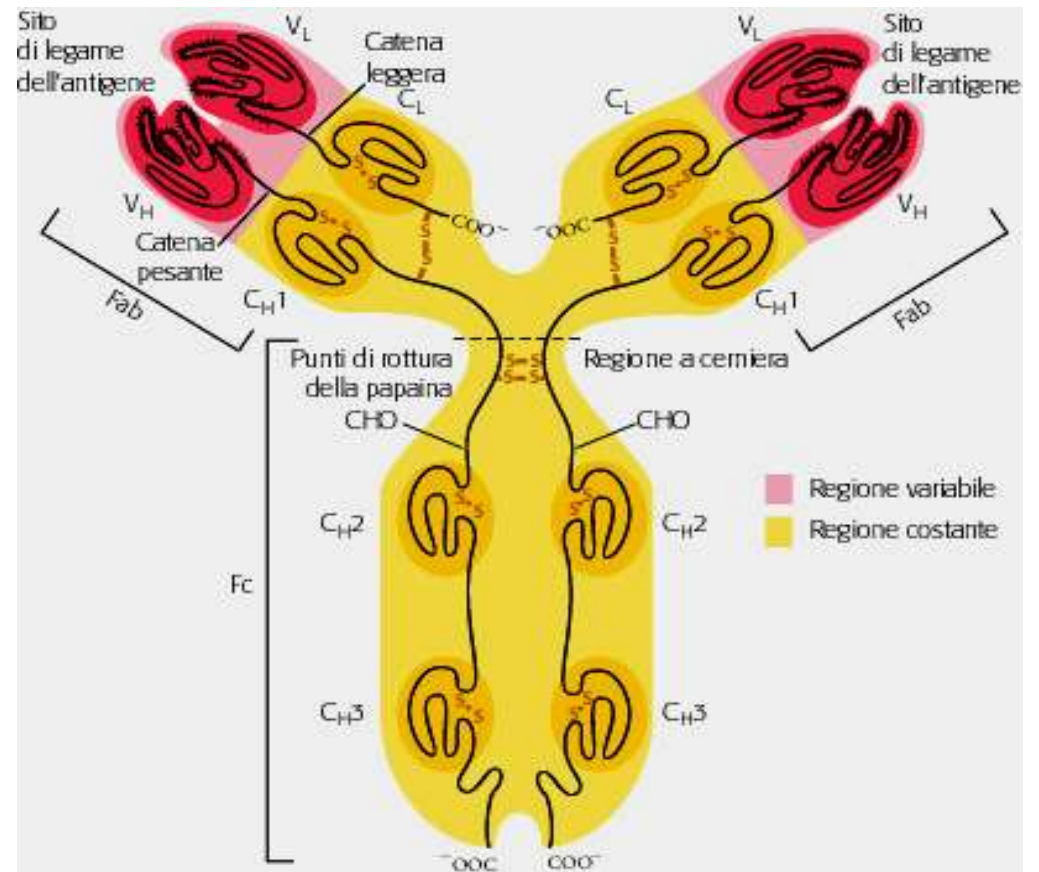
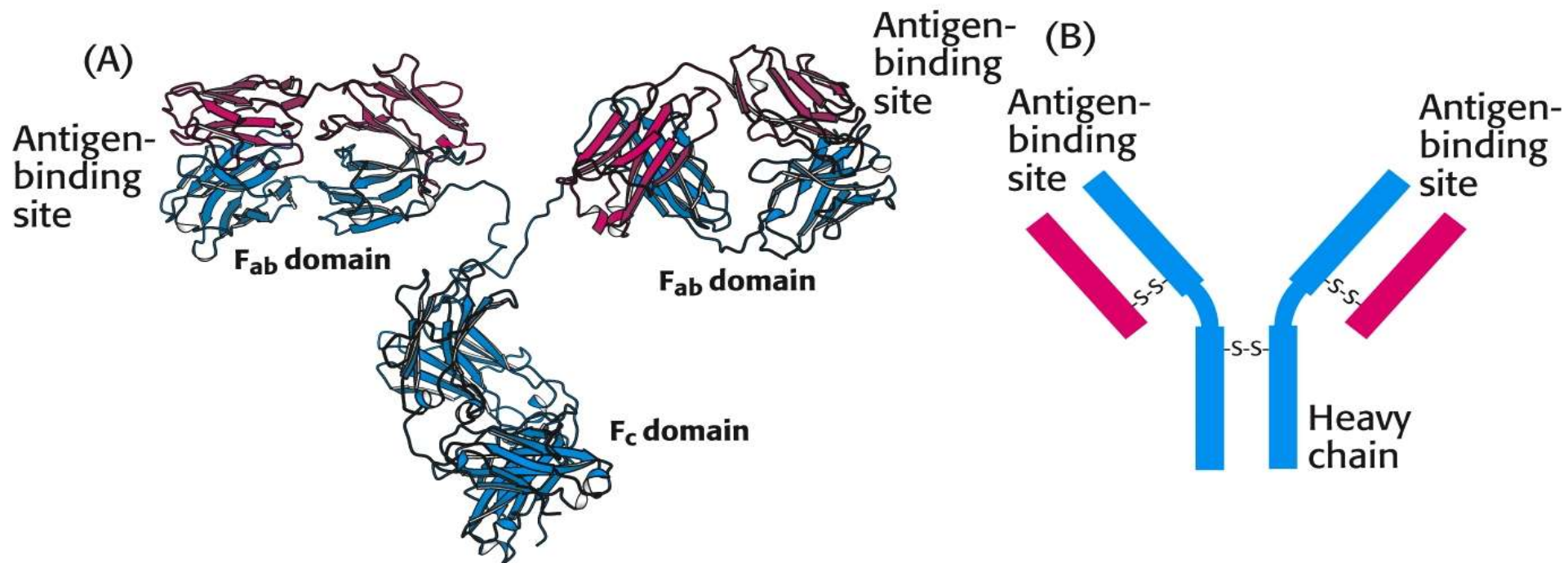
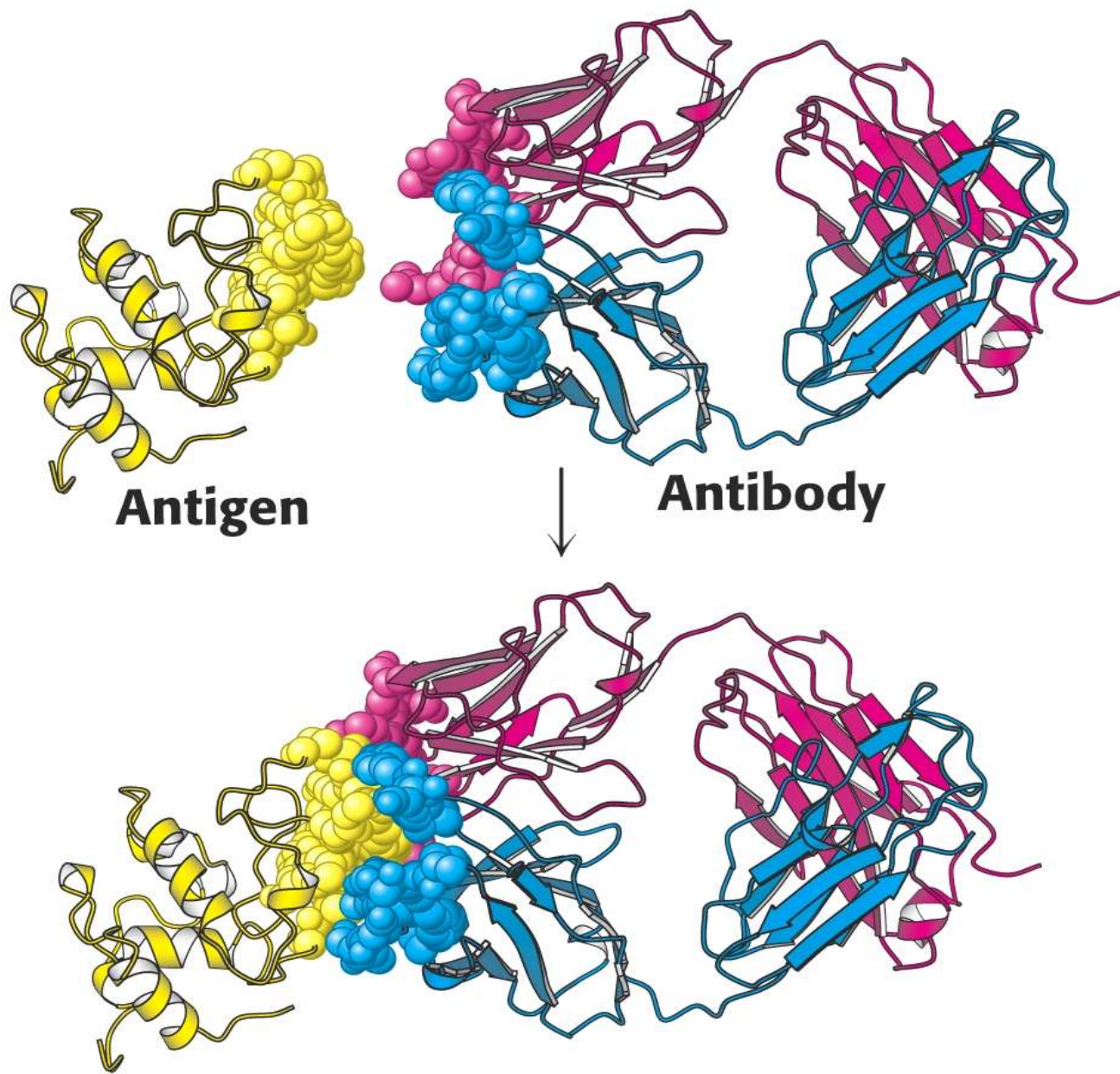


Metodi biochimici che utilizzano anticorpi

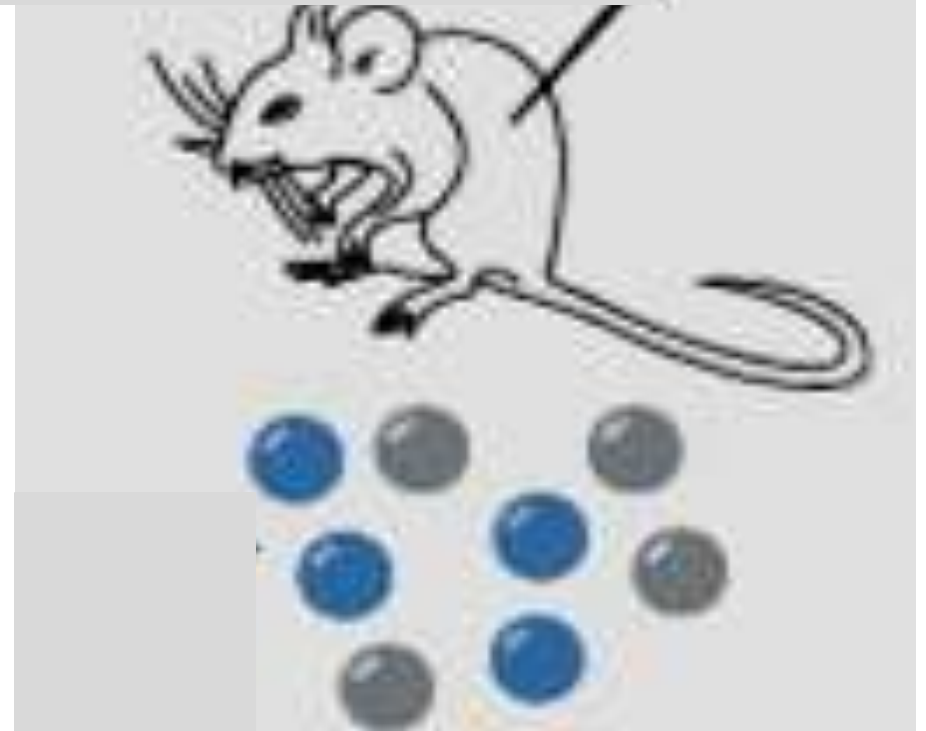




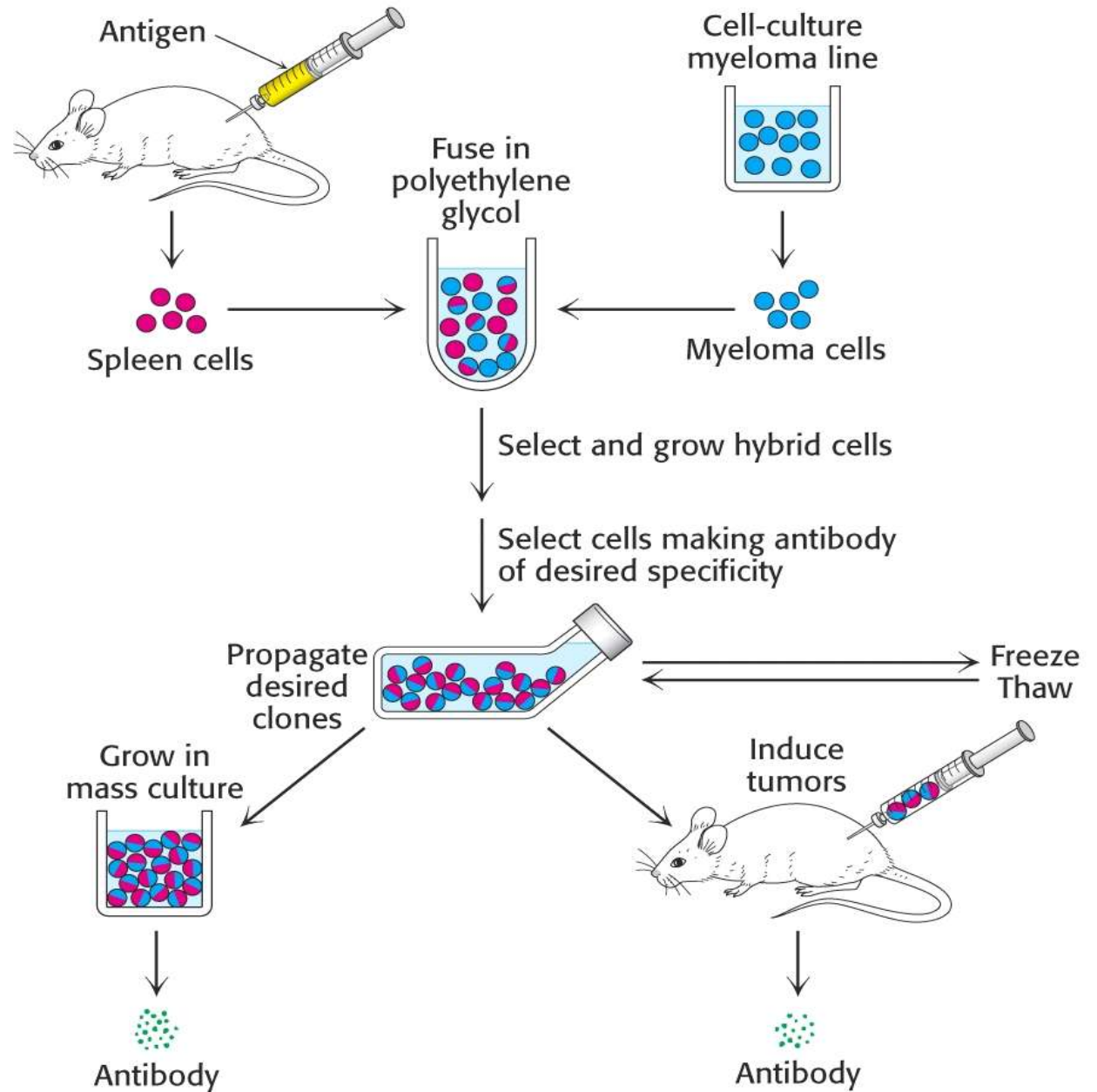


Produzione di anticorpi policlonali

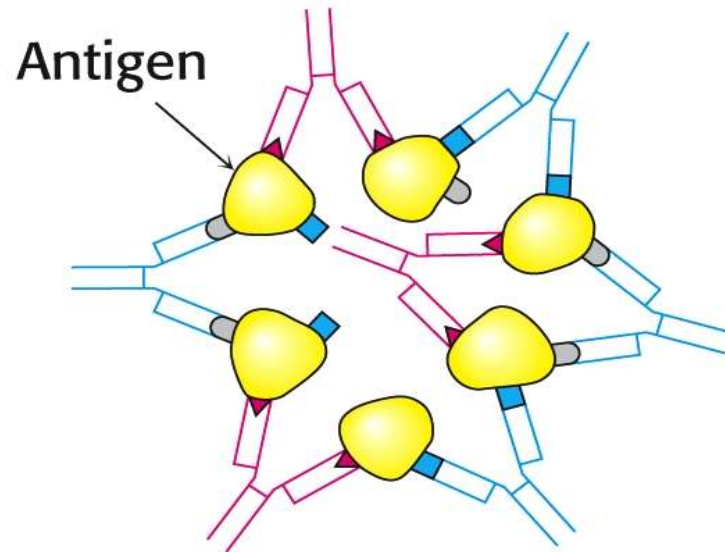
1. Si inietta nel topo (o coniglio) l'antigene X **purificato**
2. Si preleva il siero, che contiene anticorpi contro X
3. Eventualmente si purificano gli anticorpi



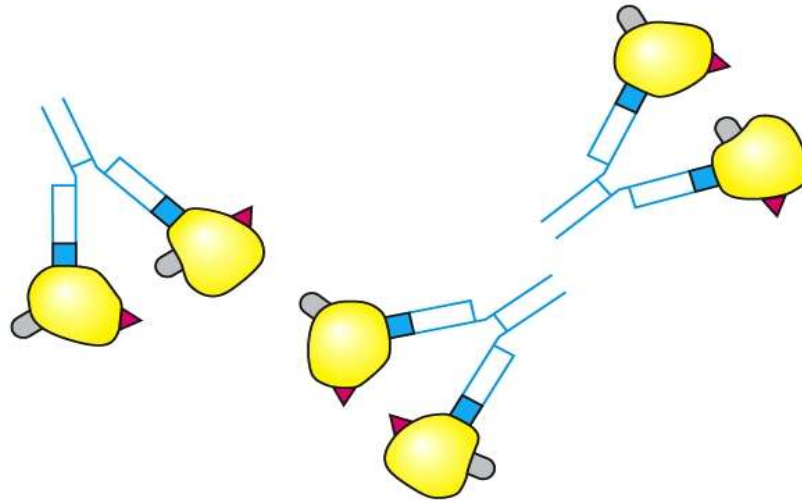
Produzione di anticorpi monoclonali



Polyclonal Antibodies



Monoclonal Antibodies

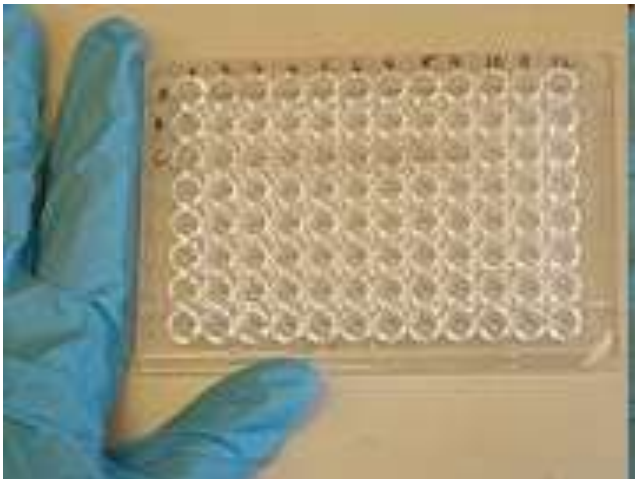


ELISA = Enzyme-Linked ImmunoSorbent Assay

E' usato in diagnostica, nel controllo di qualità, in tossicologia, nella ricerca etc.



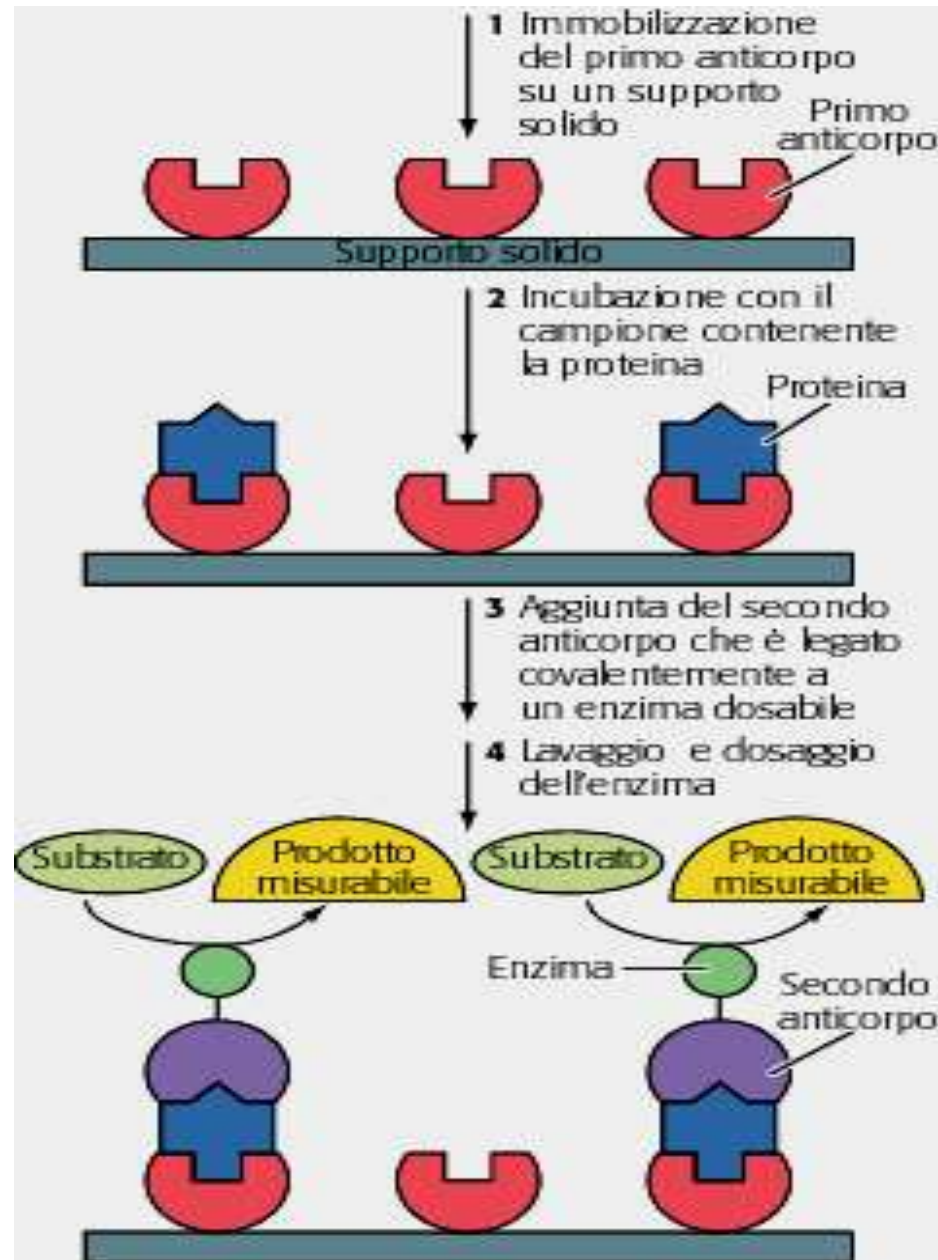
Eva Engvall (1971)



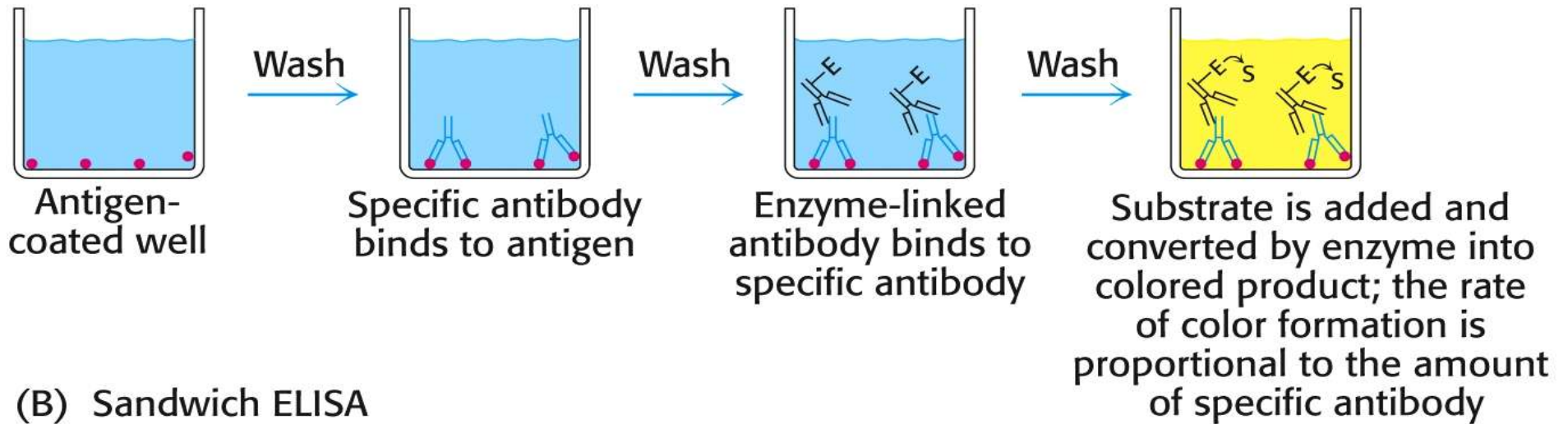
Consente di conoscere la quantità di un antigene (per il quale si disponga di un anticorpo) in un campione anche complesso (ad es sangue).

L'antigene può essere anche un'anticorpo (ad es. contro virus dell'epatite)

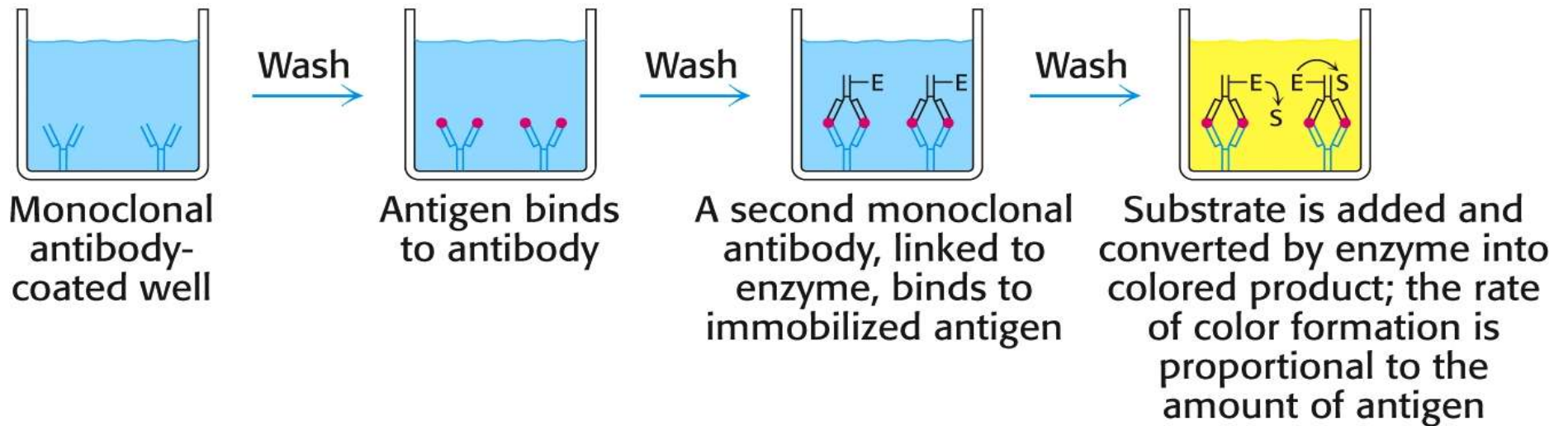
ELISA = Enzyme-Linked ImmunoSorbent Assay



(A) Indirect ELISA



(B) Sandwich ELISA



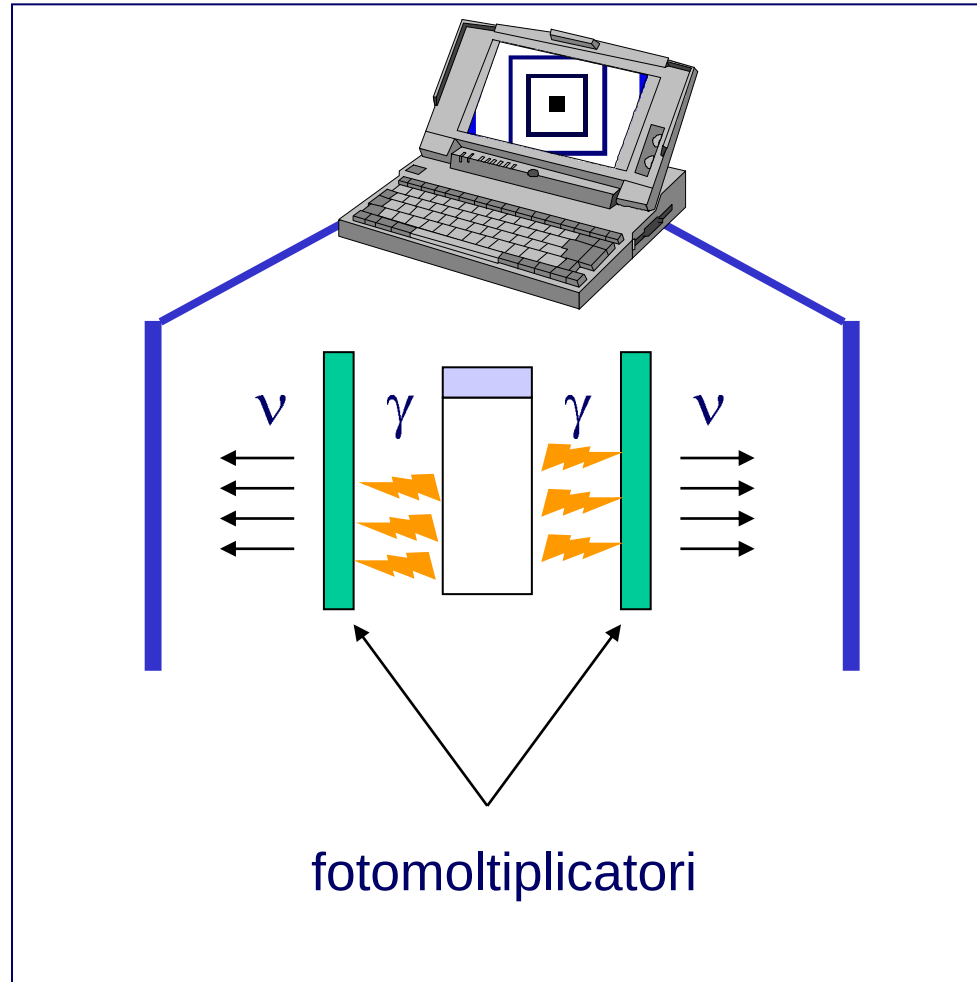
ELISA

Vantaggi : sensibile ed specifico

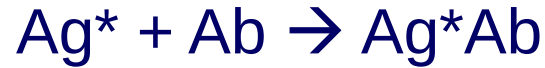
Svantaggi : richiede macchinari specialistici (lettore di piastre), costo, lineare solo in un range limitato, richiede buoni controlli interni.

RIA = Radio-Immuno Assay

Rosalyn Yalow and Solomon Aaron Berson in the 1950s.



RIA = Radio-Immuno Assay



Misura di Ag^* libero

Misura di Ag

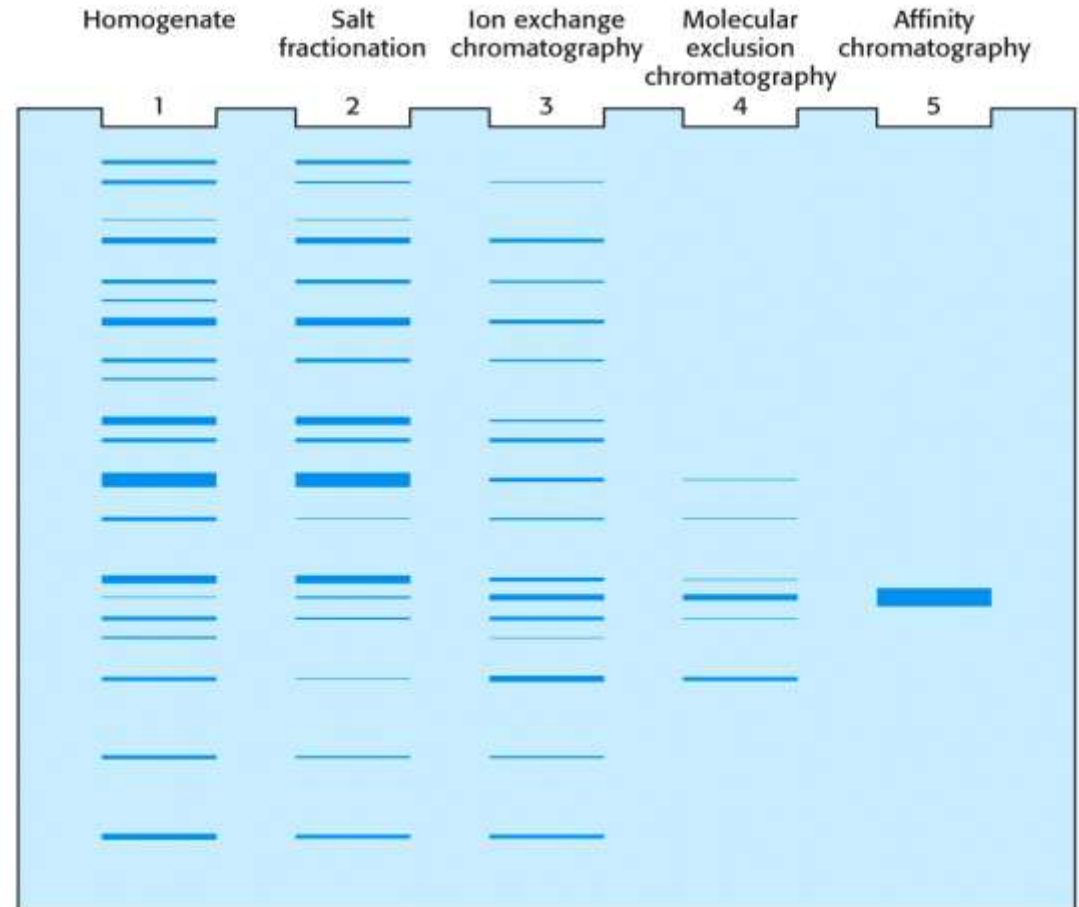
Vantaggi : estremamente sensibile ed estremamente specifico

Svantaggi : richiede macchinari specialistici (contatore di radiazioni γ), costo, potenzialmente rischioso per l'operatore.

Western blot.1

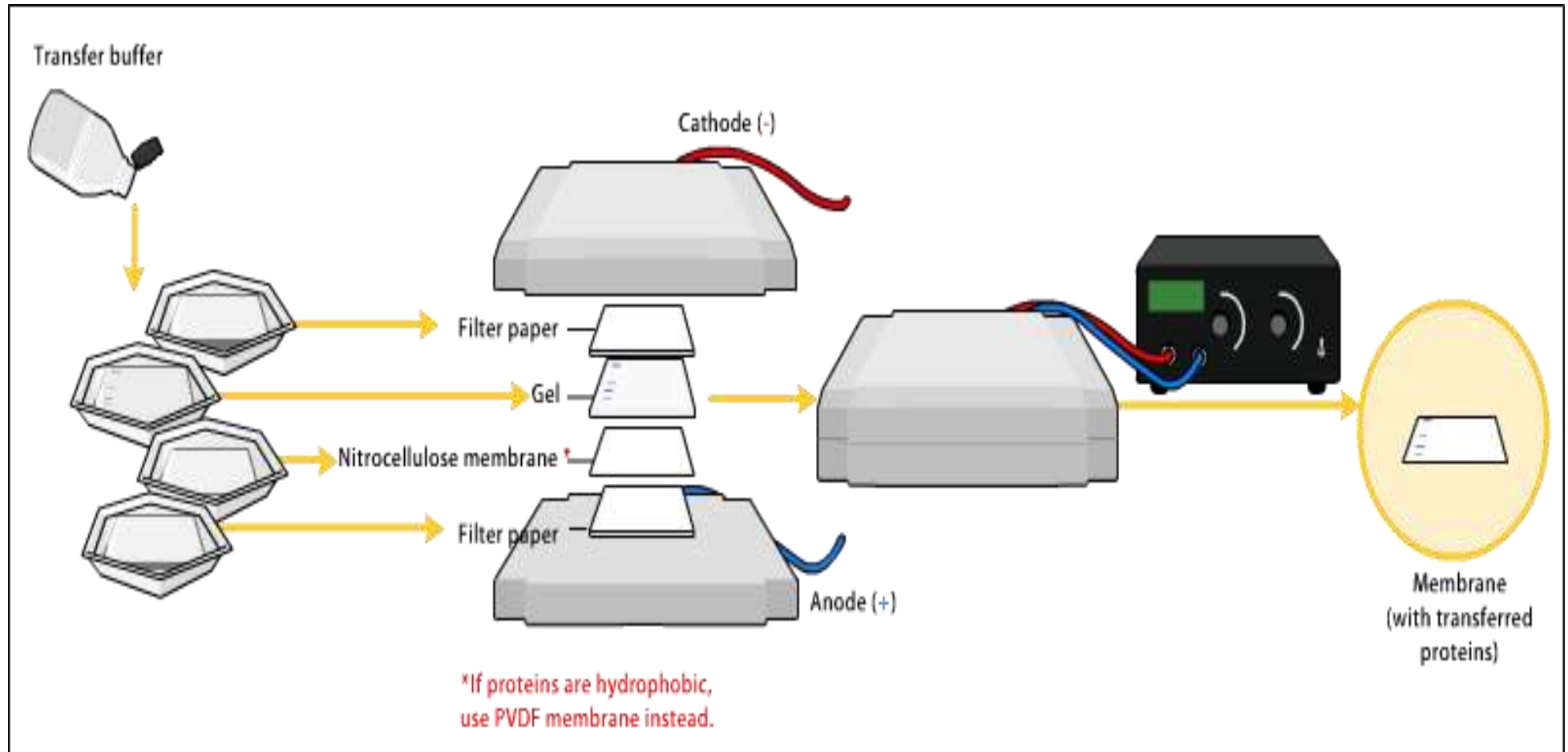
Individuazione e stima di proteine anche da miscele complesse

1. Preparazione del campione (ad es. omogenato di tessuto)
2. Gel elettroforesi (di solito SDS-PAGE)



Western blot. 2

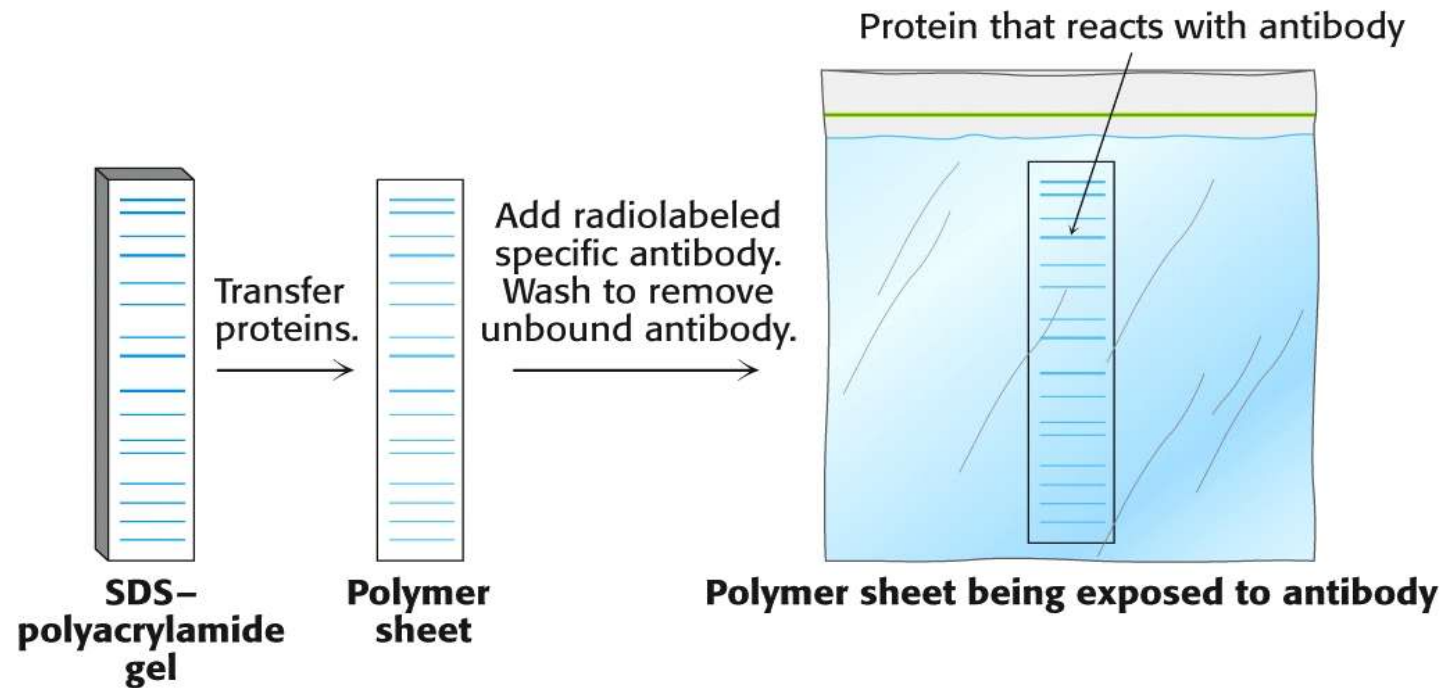
3. Trasferimento delle proteine dal gel ad una membrana (nitrocellulosa o PVC)



Western blot. 3

4. Saturazione di siti di legame non specifici

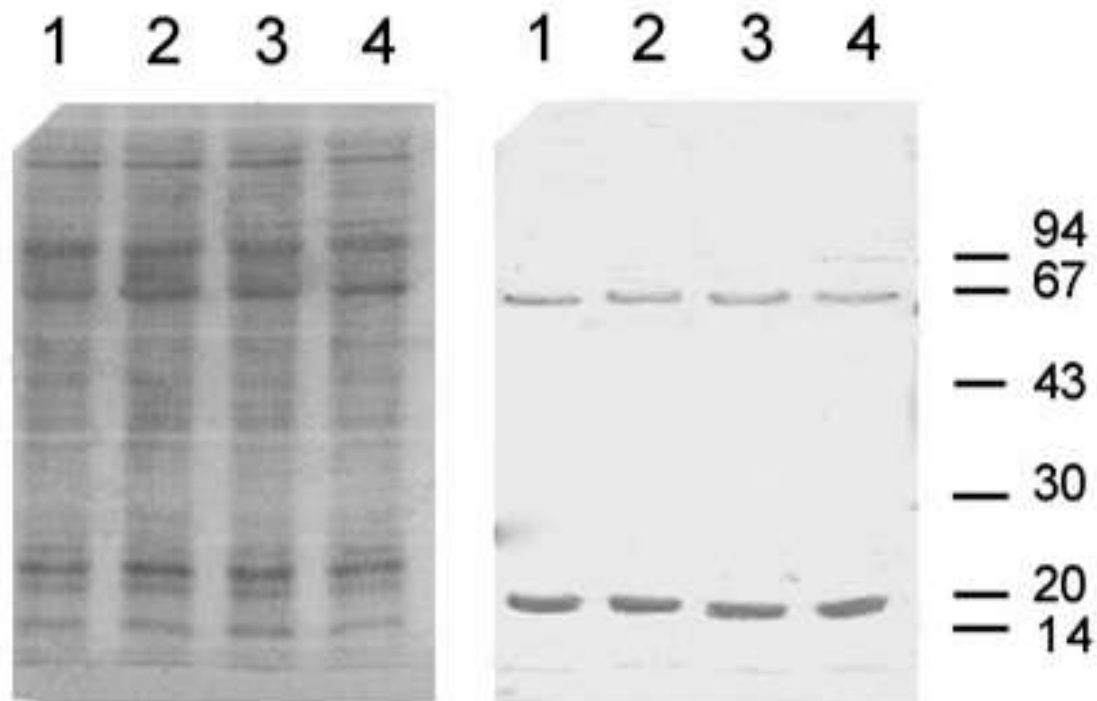
5. Incubazione con anticorpo primario



Western blot. 4

6. Lavaggio ed incubazione con un anticorpo secondario anti-IgG

7. Detection



7. Detection

1. L'anticorpo secondario è legato ad un enzima che produce una sostanza :

- Colorata
- Luminescente
- Fluorescente



Sistema di visualizzazione ed analisi
(densitometria)

ECL = Enzymatic ChemiLuminescence



Ad es. in presenza di un opportuno catalizzatore :

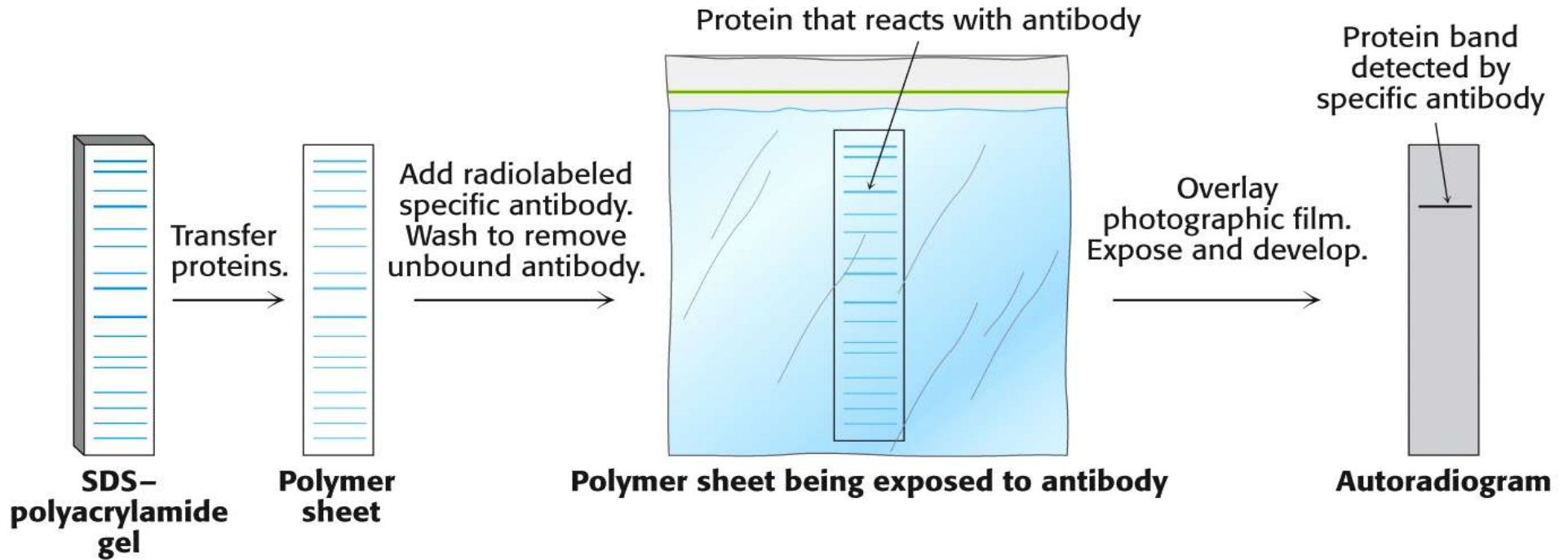


In cui :

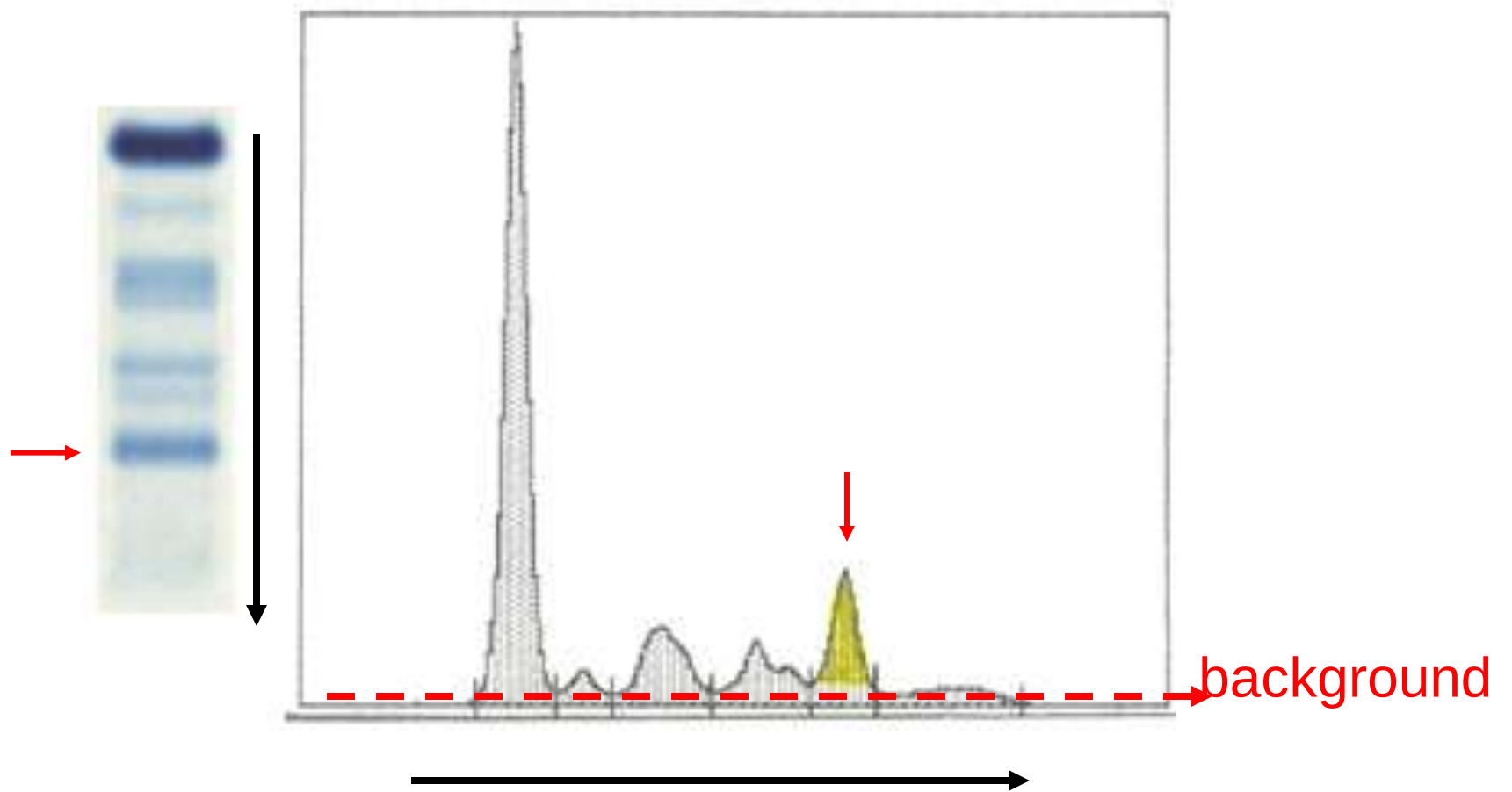
- * 3-APA è il 3-aminophthalate
- * 3-APA[$\diamond$] è lo stato eccitato fluorescente.

7. Detection

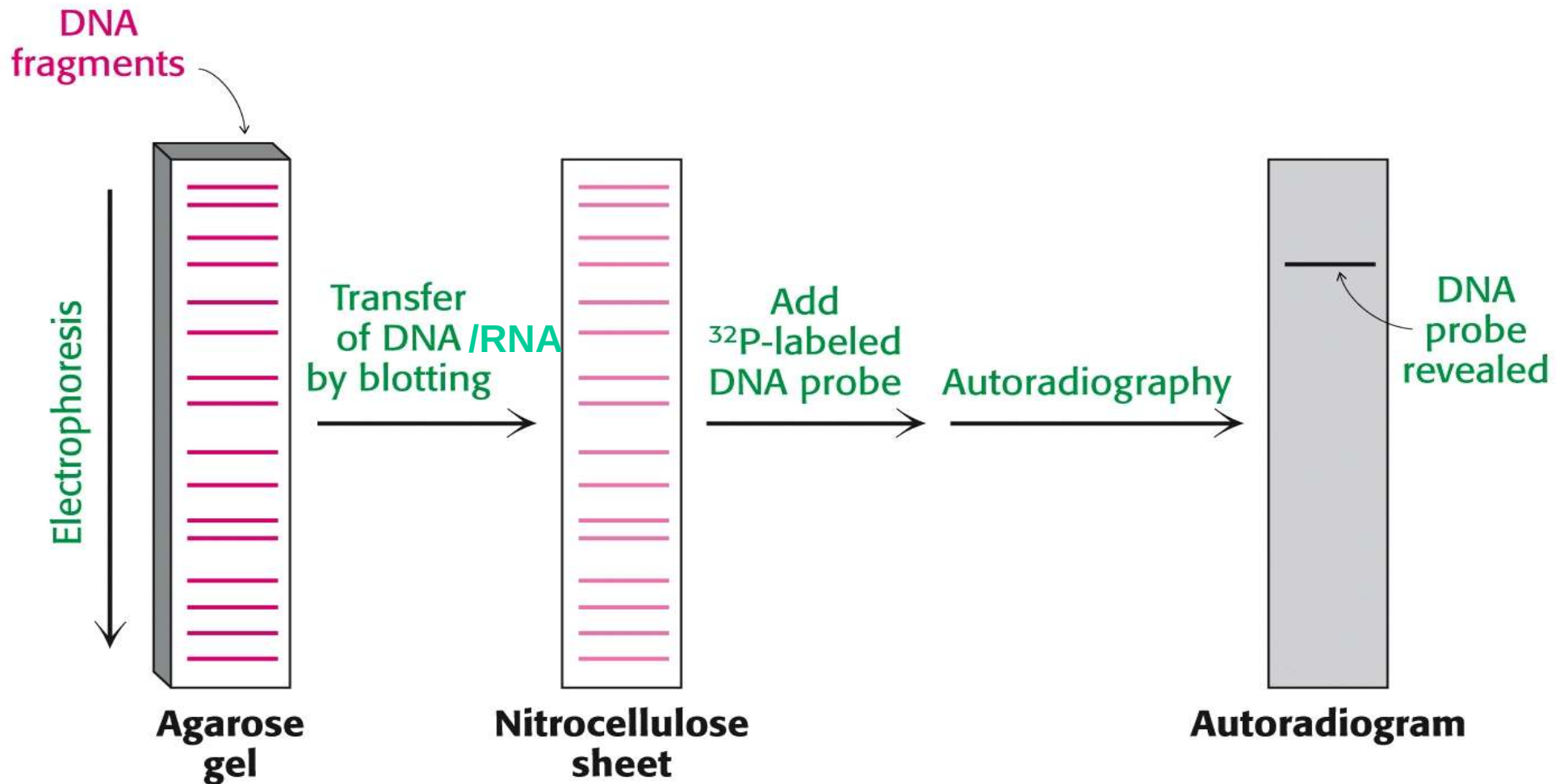
2. L'anticorpo secondario è marcato con un isotopo radioattivo



Quantitation



Southern/Northern blot.1



Cromatografia **per affinità**

Fase stazionaria : granuli di resina coniugata al ligando

Fase mobile: solventi acquosi

Uso comune: separazione di proteine



molecola A



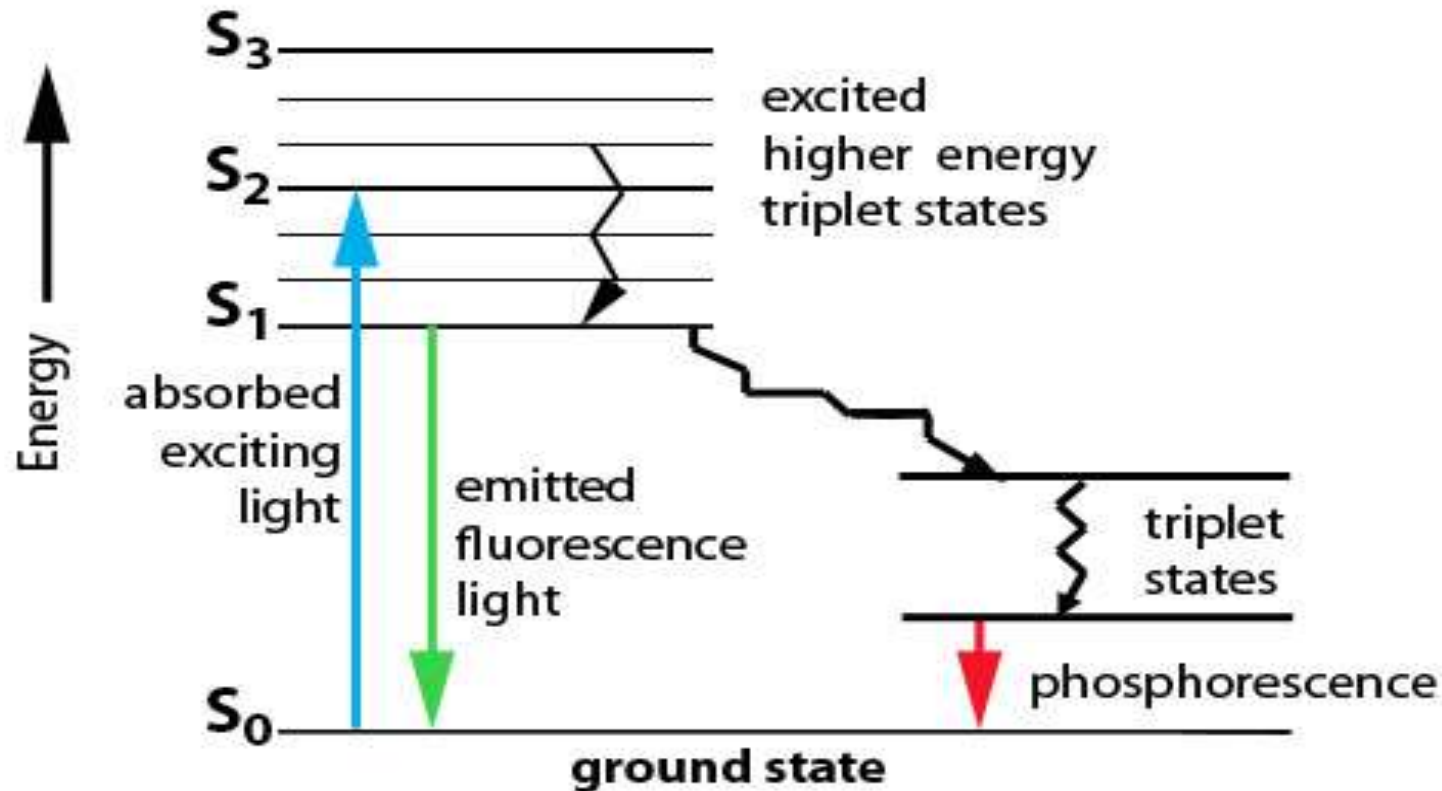
Ligando specifico
per la molecola A



Altre molecole
non affini al ligando

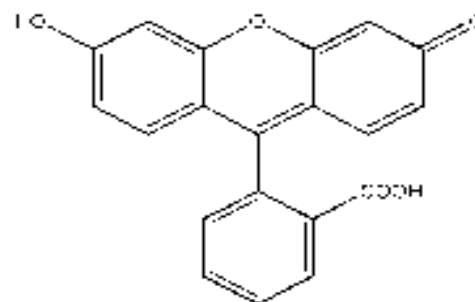
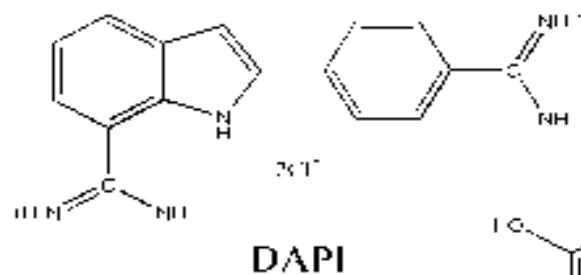
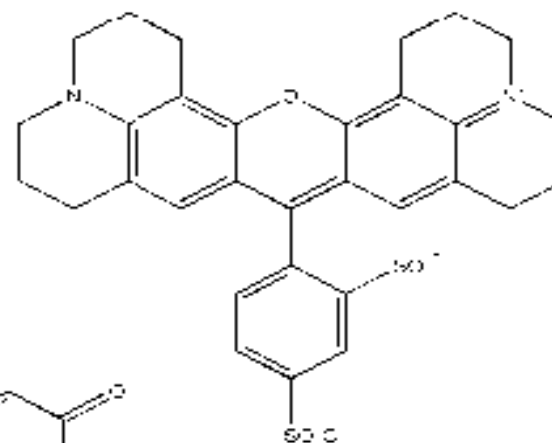
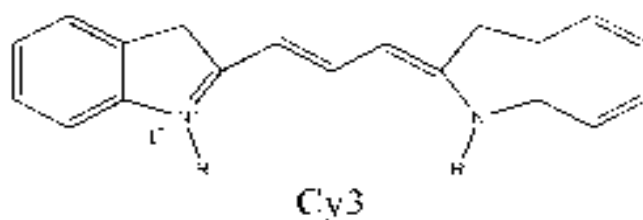
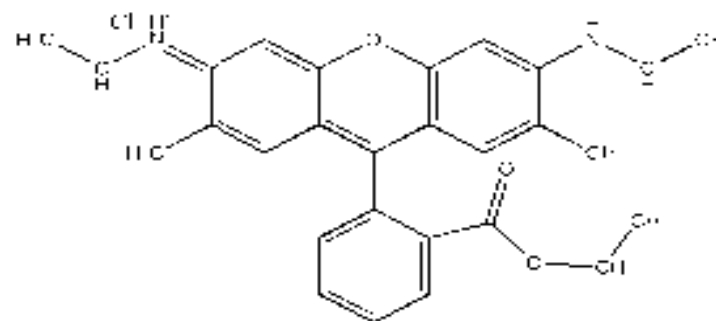
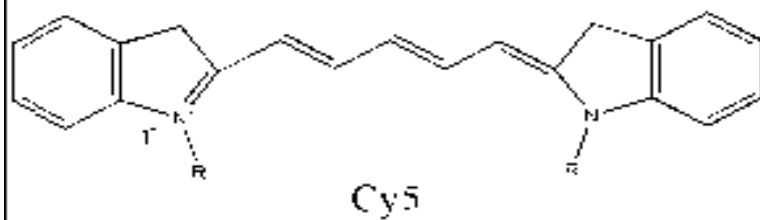


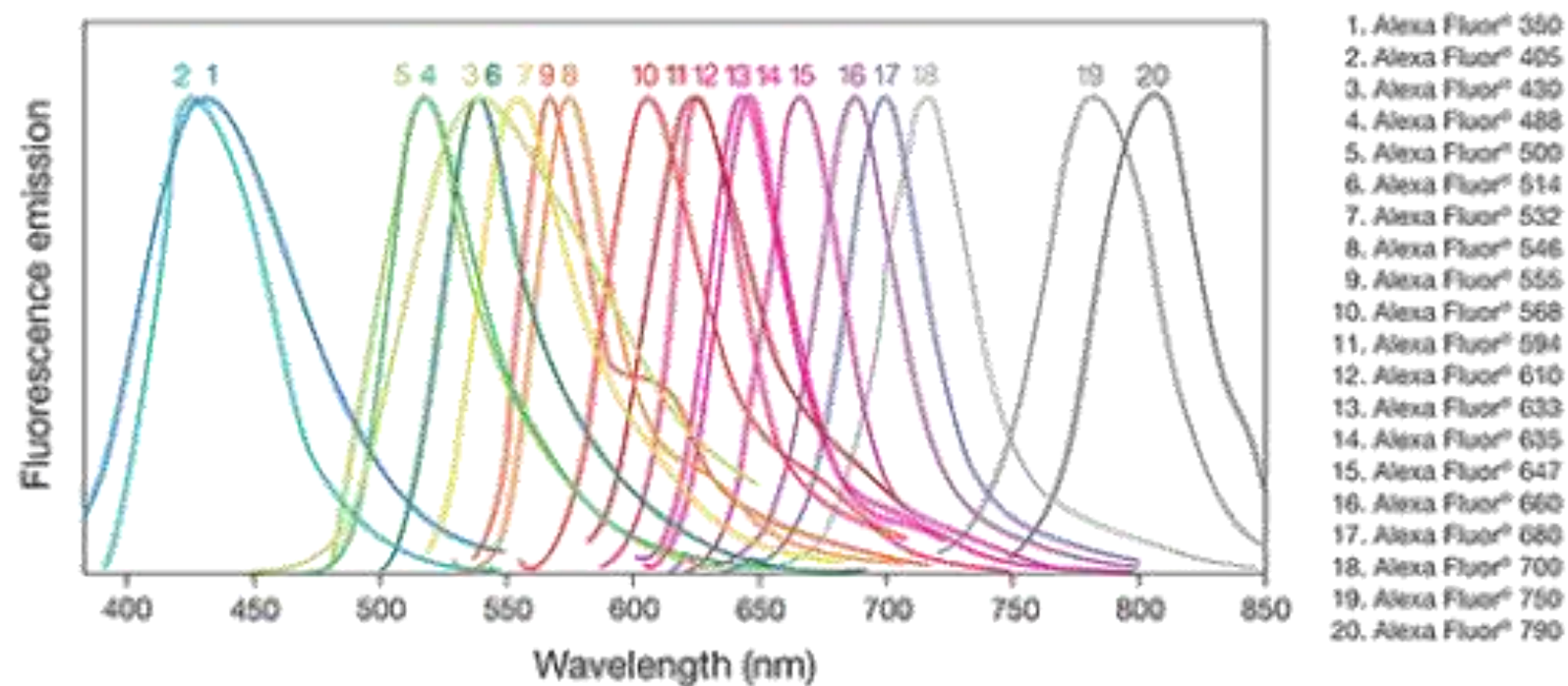
Metodi che utilizzano anticorpi legati a fluorocromi



I diagrammi di Jablonski riportano convenzionalmente la struttura vibronica (elettronica e vibrazionale) degli stati elettronici di una molecola, espressi in termini di energia rispetto alle coordinate nucleari

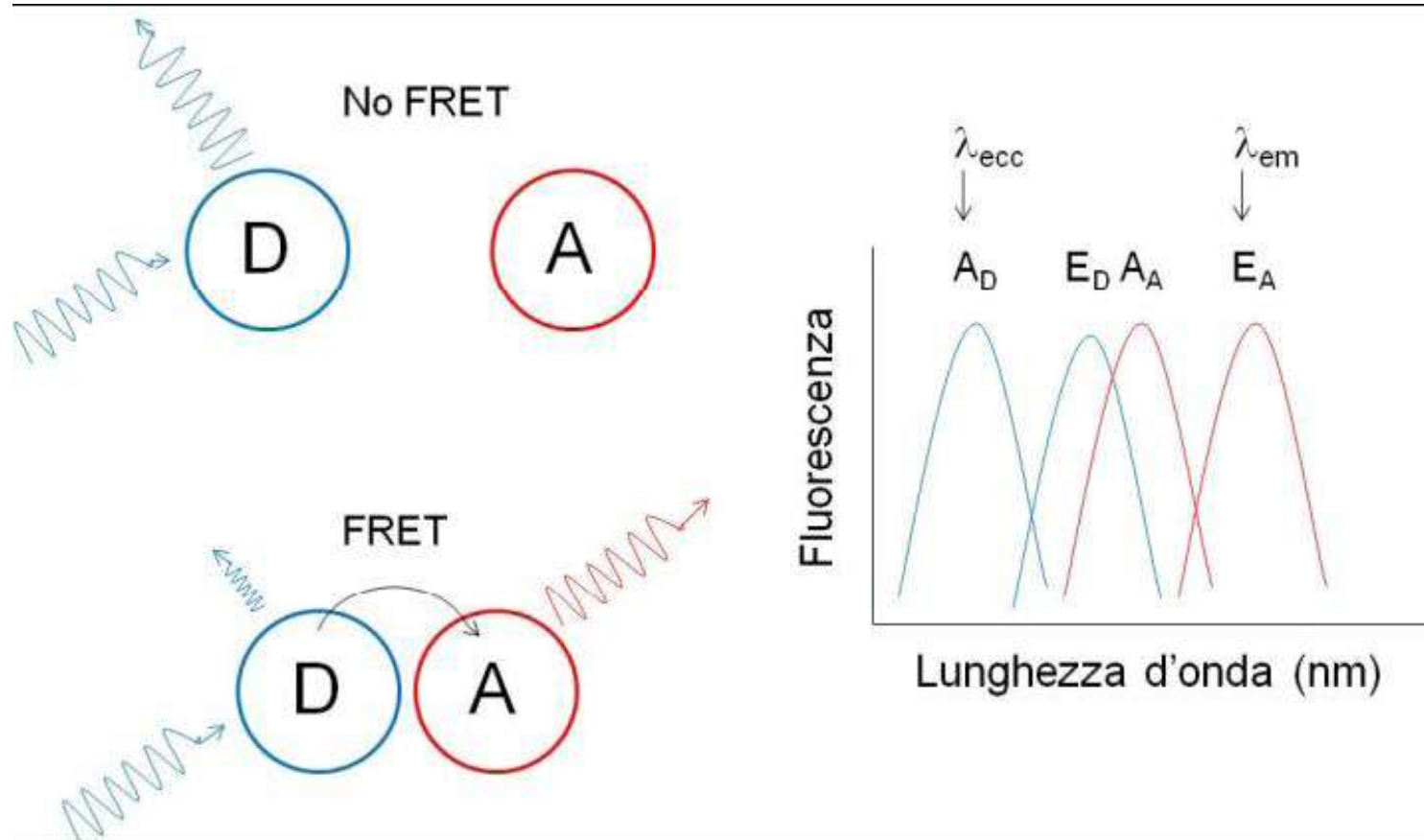
Strutture di comuni fluorocromi



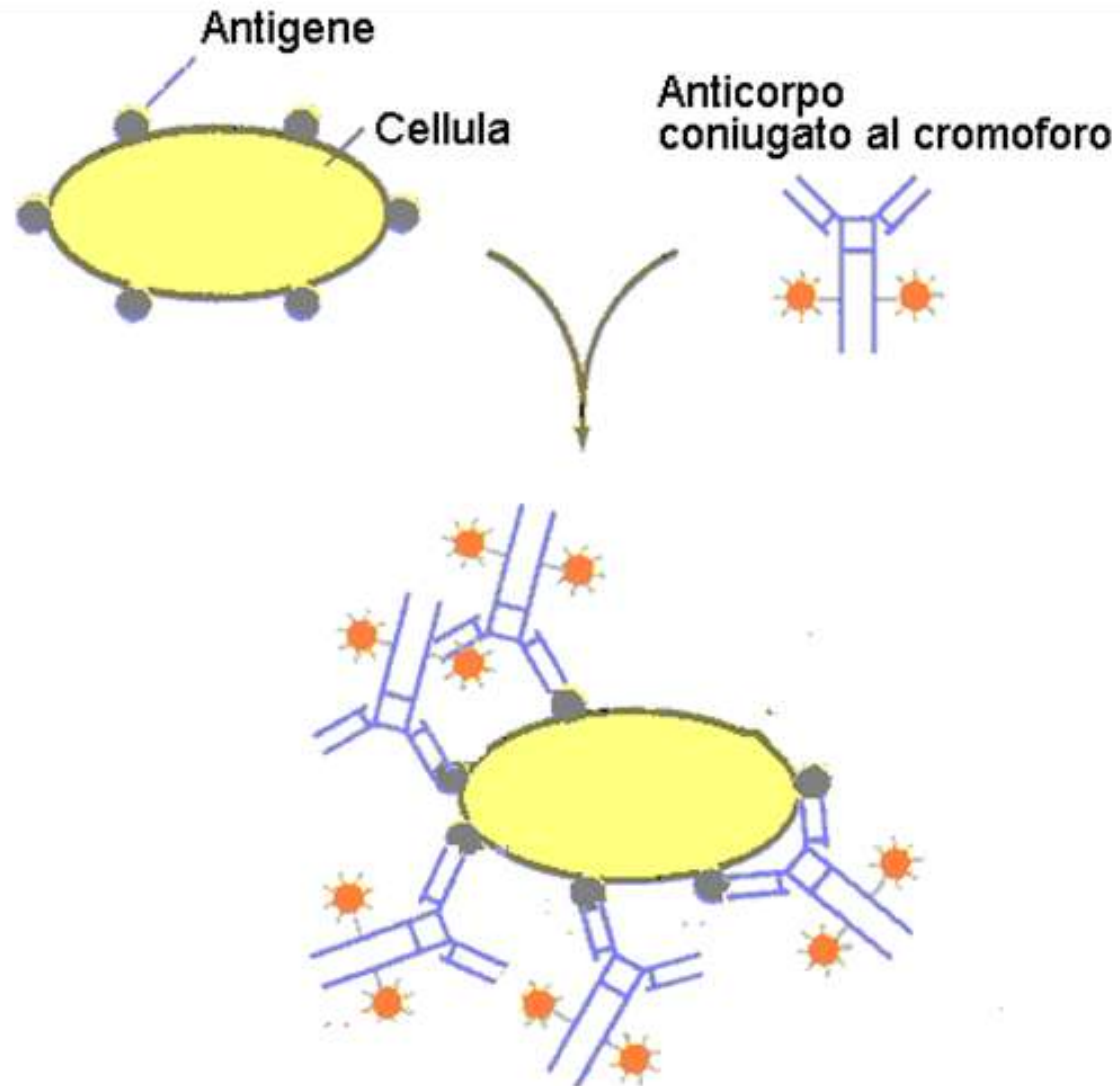


Fluorescence Resonance Energy Transfer (FRET)

La FRET è un meccanismo di quenching dinamico in cui si verifica un **trasferimento di energia da un fluoroforo donatore che si trova nello stato eccitato a un differente fluoroforo accettore**



CITOMETRIA A FLUSSO



CITOMETRIA A FLUSSO

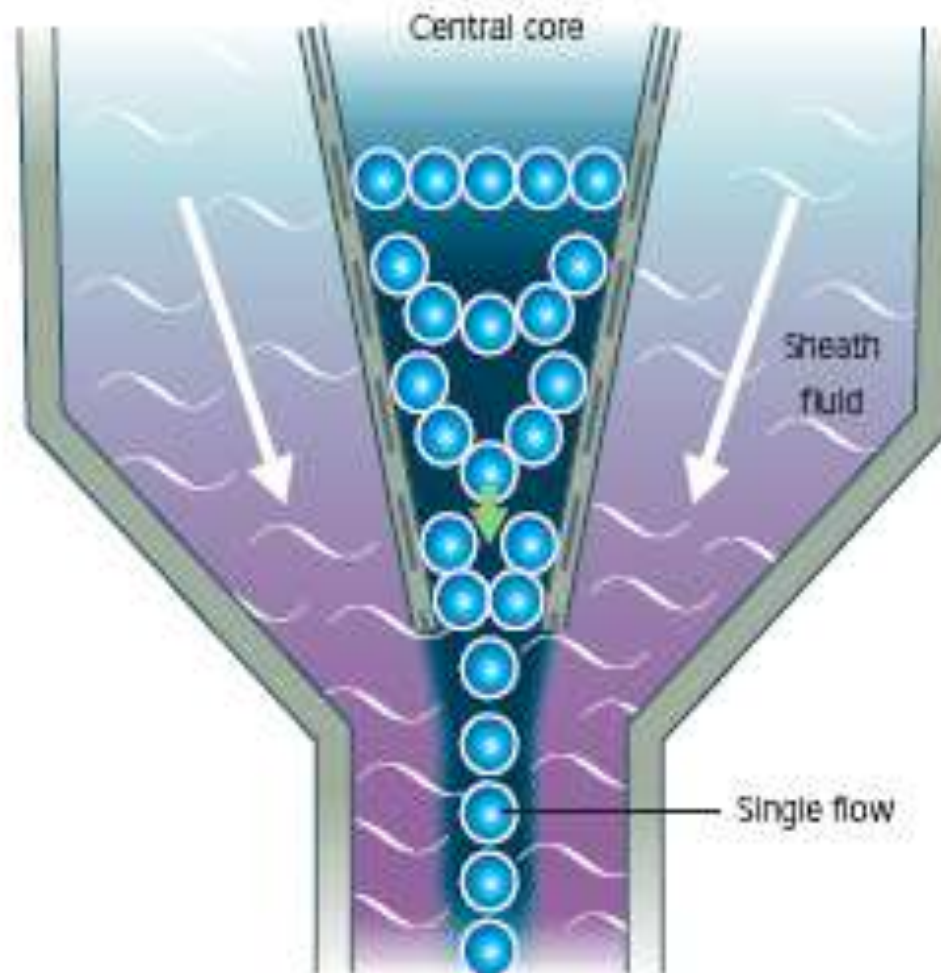
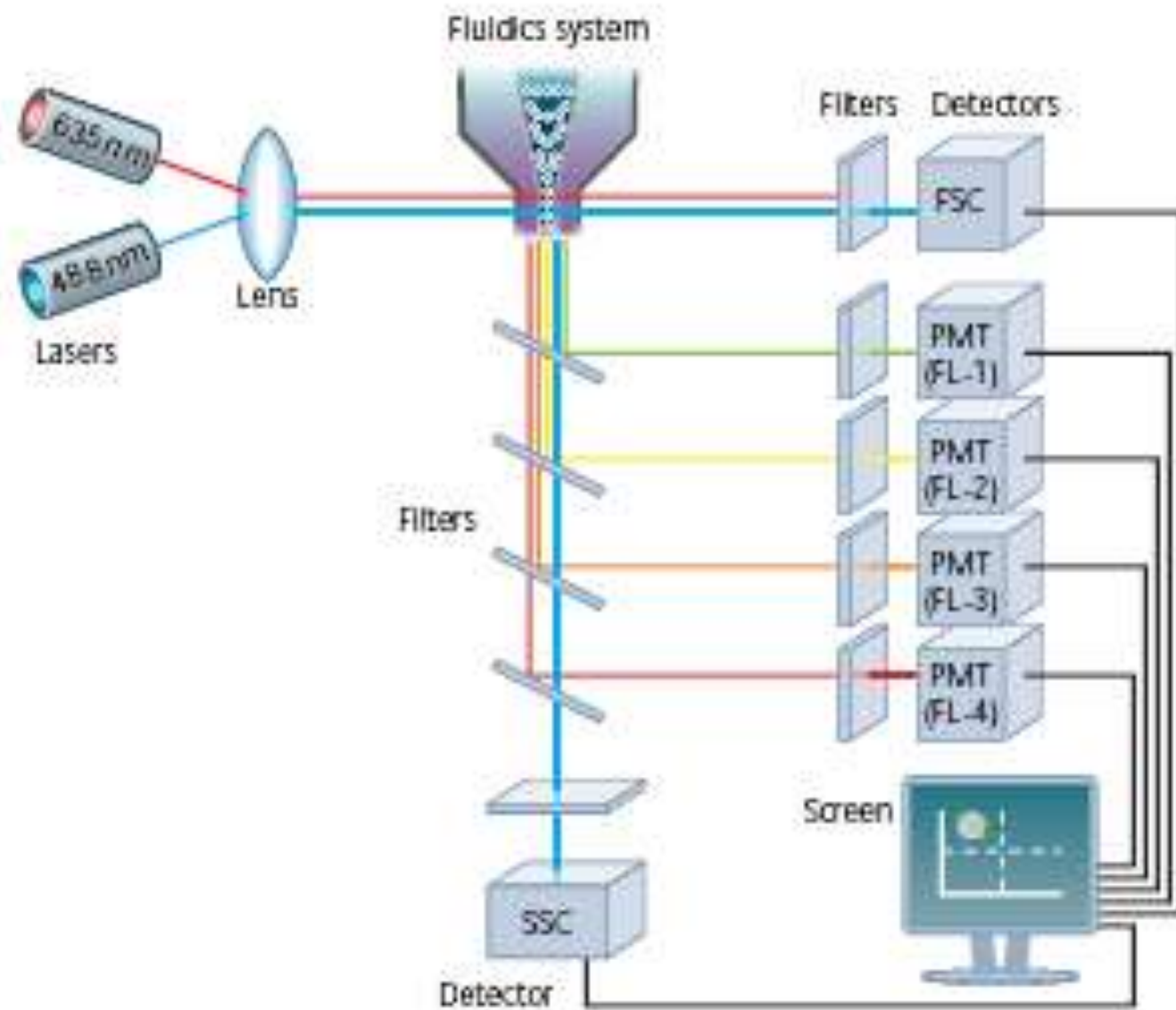
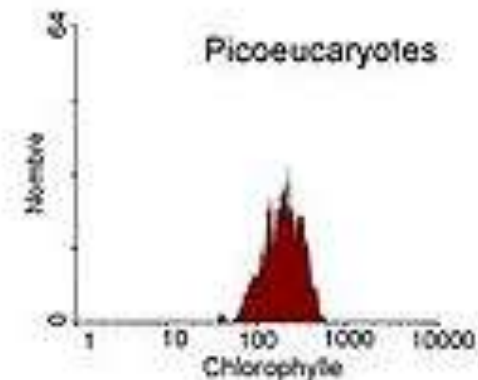
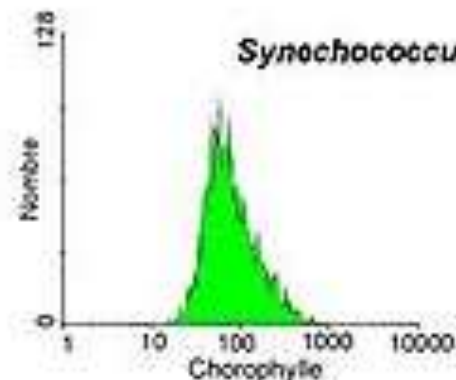
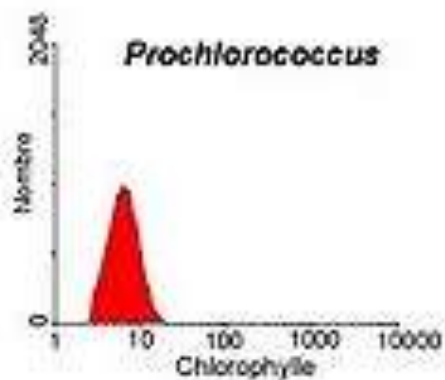
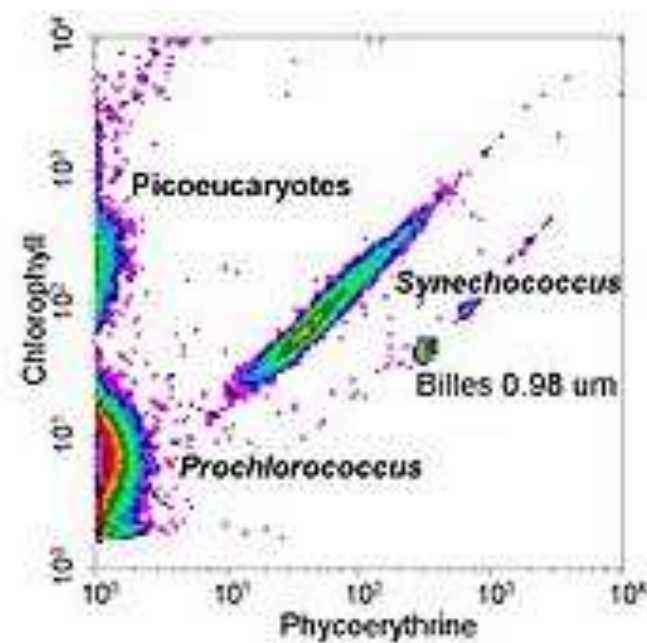
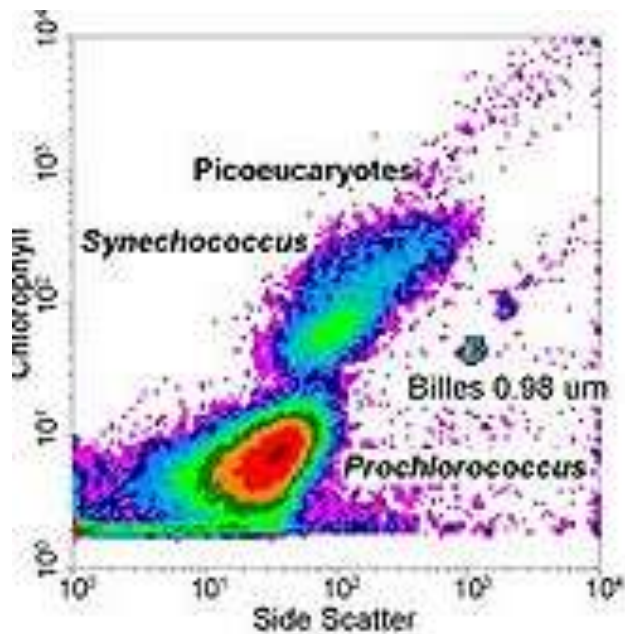


FIGURE 1

Hydrodynamic focusing produces a single stream of particles

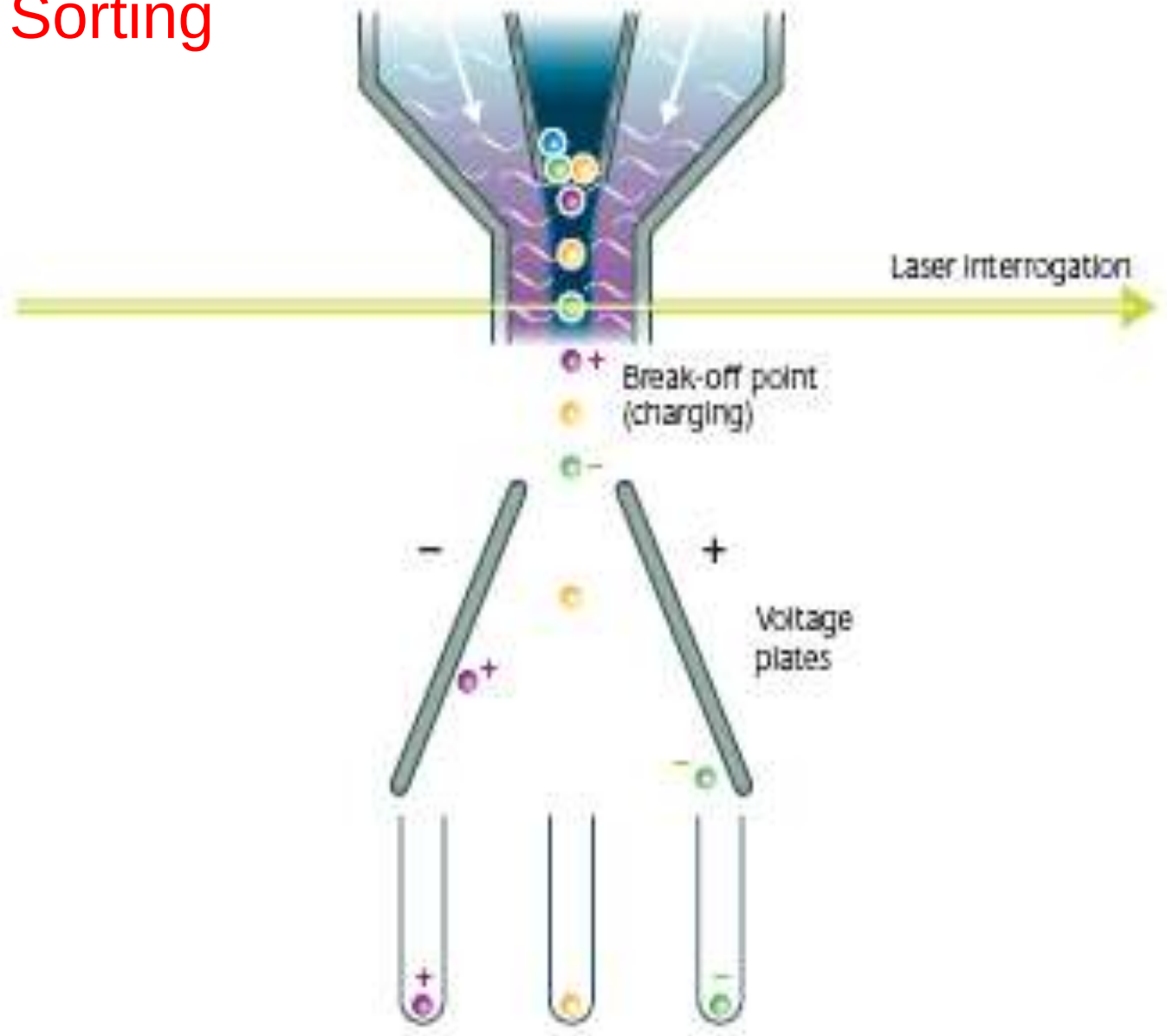
Schema di un citoflurimetro a flusso





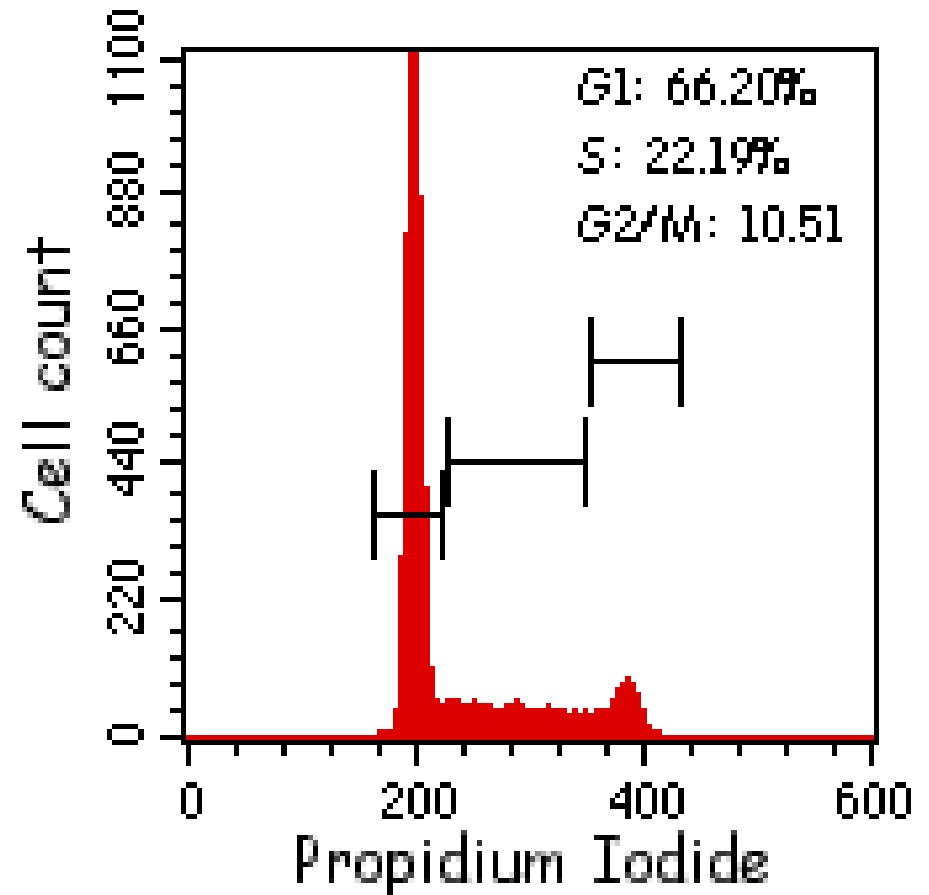
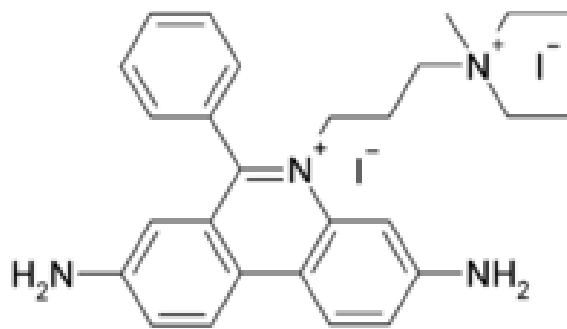
Analysis of a marine sample of photosynthetic picoplankton by flow cytometry showing three different populations (*Prochlorococcus*, *Synechococcus*, and picoeukaryotes)

Electrostatic Cell Sorting



Electrostatic flow sorting

Studio del ciclo cellulare mediante colorazione con ioduro di propidio e citometria a flusso



Lo ioduro di propidio colora il DNA intercalandosi tra le basi
→ fluorescenza

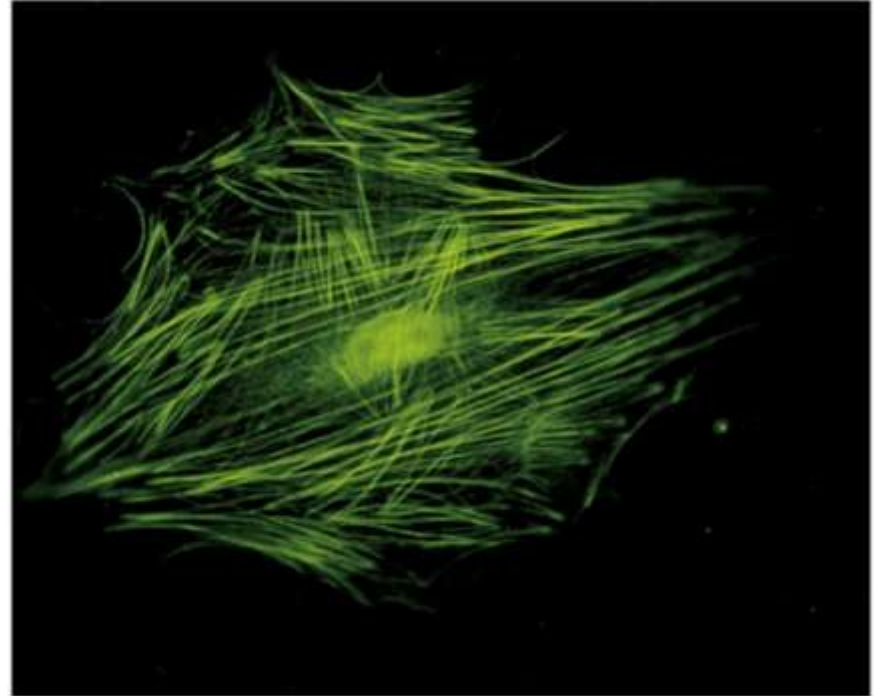
CITOMETRIA A FLUSSO

Vantaggi : estremamente sensibile ed estremamente specifico

Svantaggi : richiede macchinari specialistici, costo.

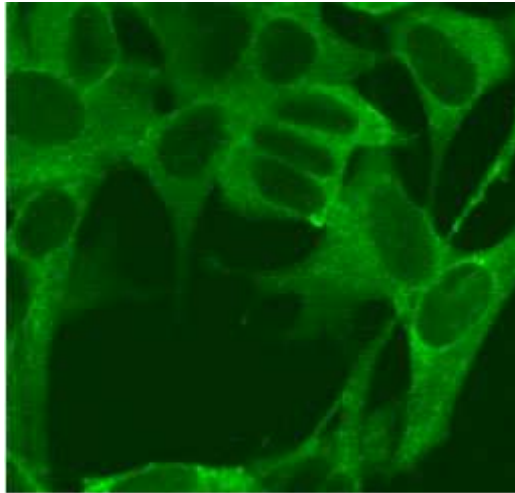
Immunoistochimica

Colorazione di un tessuto o di cellule con un anticorpo primario che riconosce specificamente una proteina. Visualizzazione in microscopia a fluorescenza mediante un anticorpo secondario coniugato ad un fluorocromo

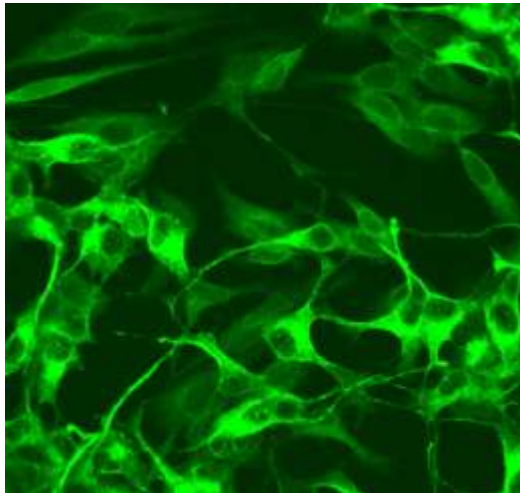
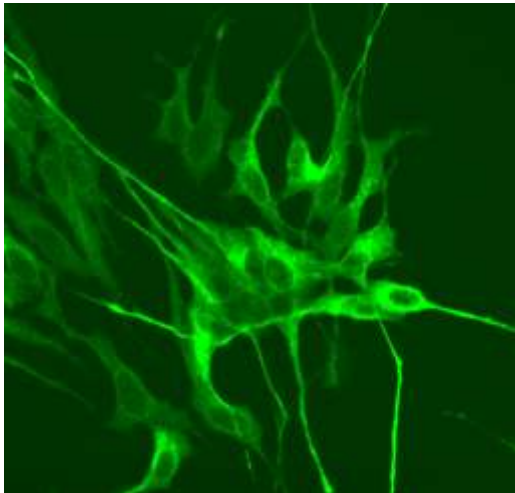
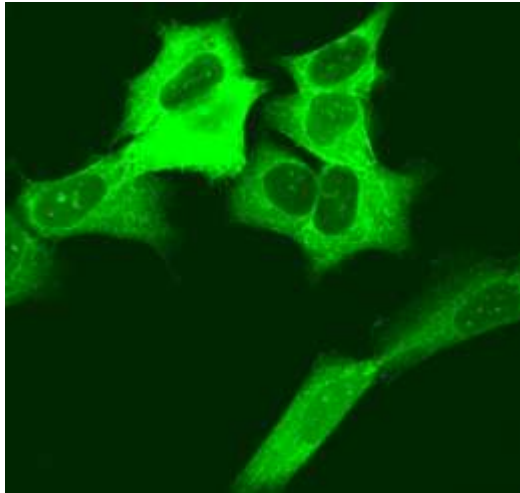


L'immunoistochimica è semi-quantitativa

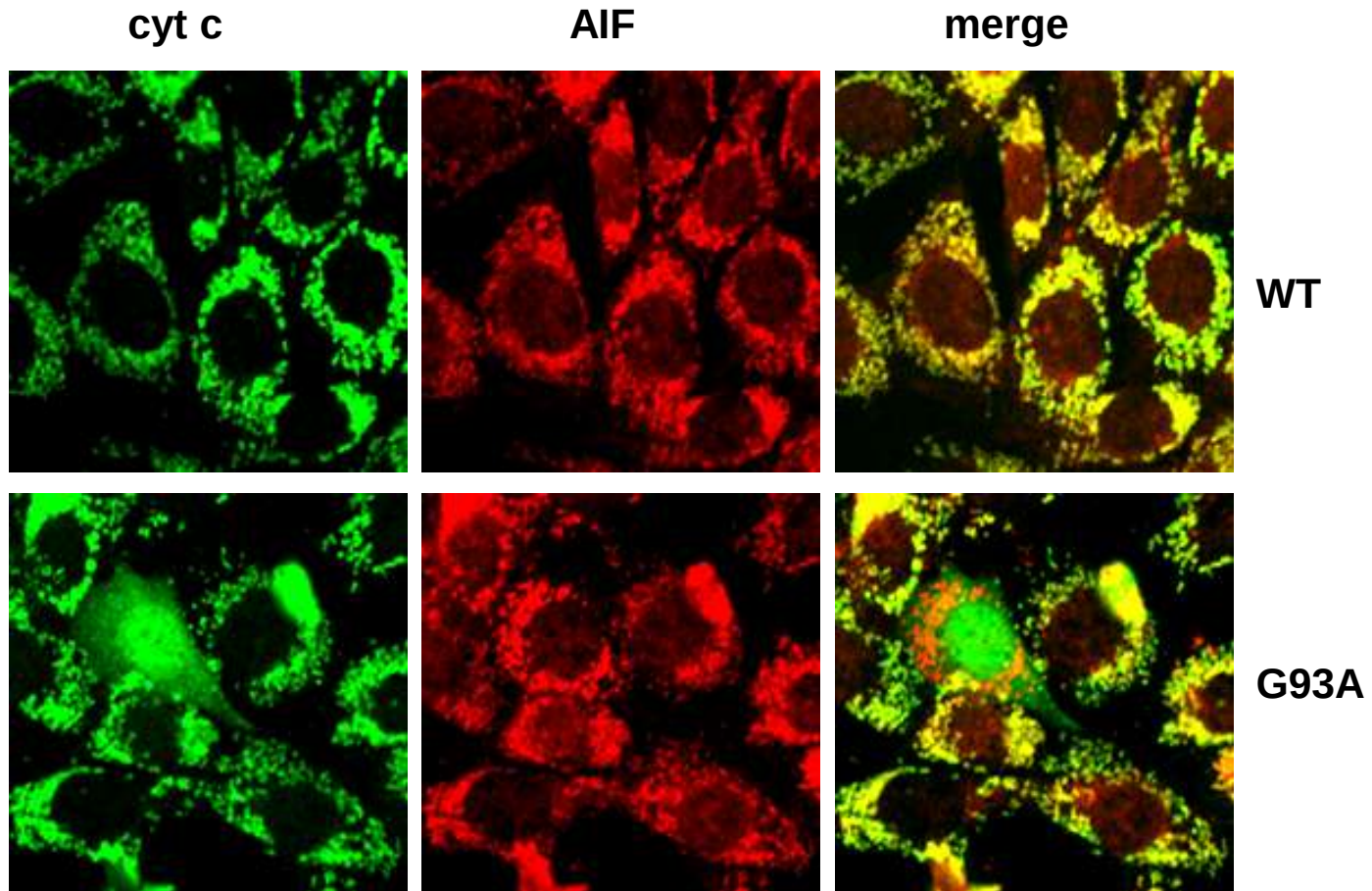
SH



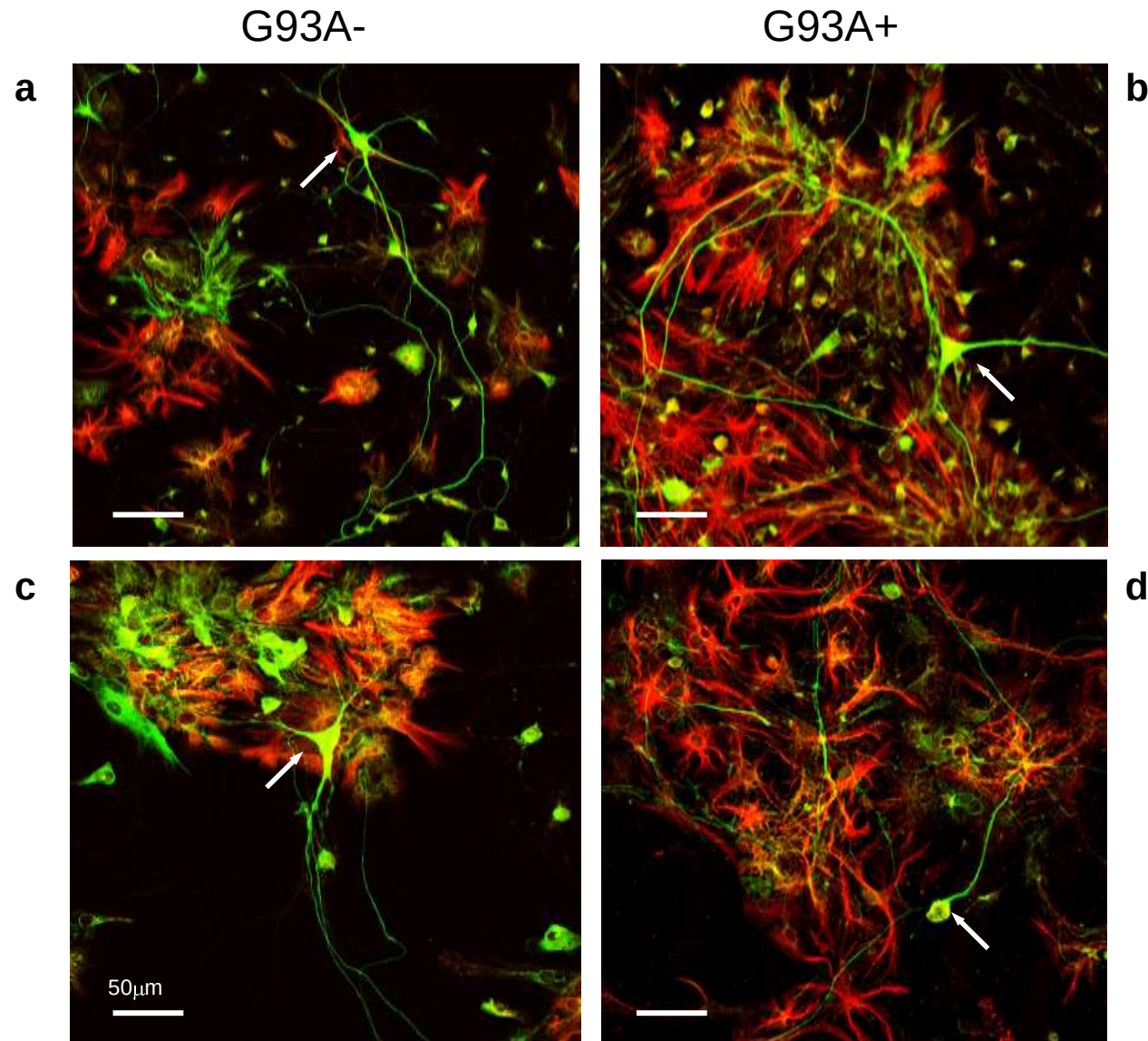
WT



L'immunoistochimica consente anche (co-)localizzazioni



L'immunoistochimica consente di differenziare tipi cellulari



Immunoistochimica in microscopia elettronica

Colorazione di un tessuto o di cellule con un anticorpo primario che riconosce specificamente una proteina. Visualizzazione in microscopia elettronica mediante un anticorpo secondario coniugato a particelle di oro colloidale (elettrondense)

