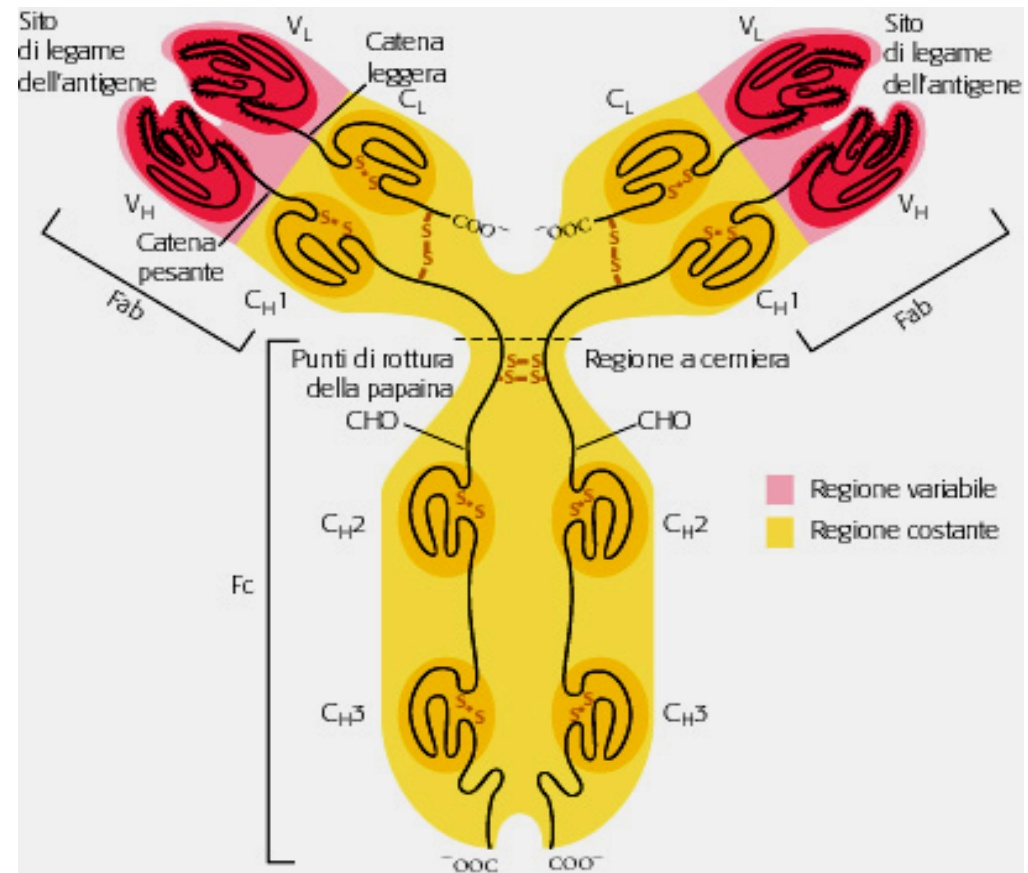
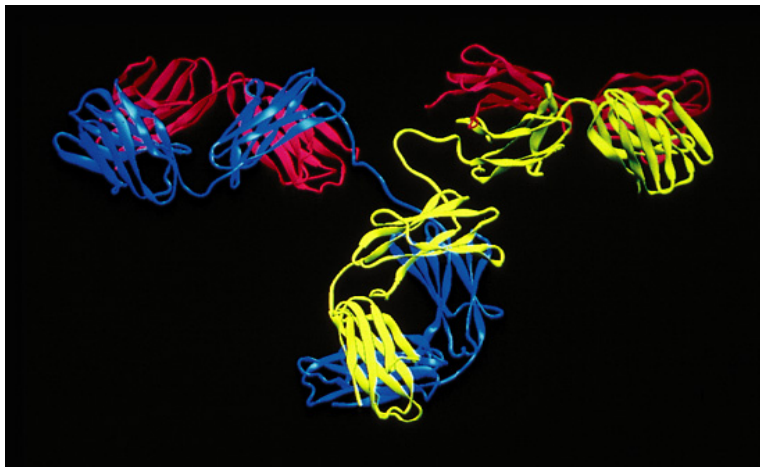


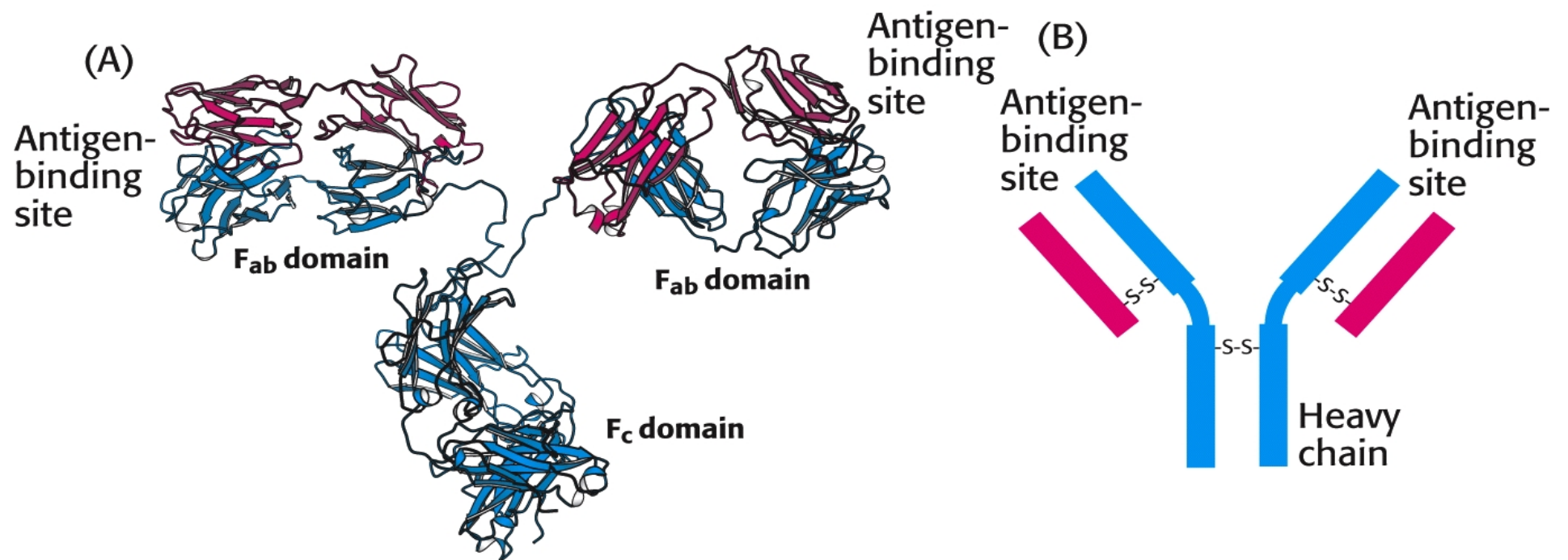
ATTENZIONE !!!!

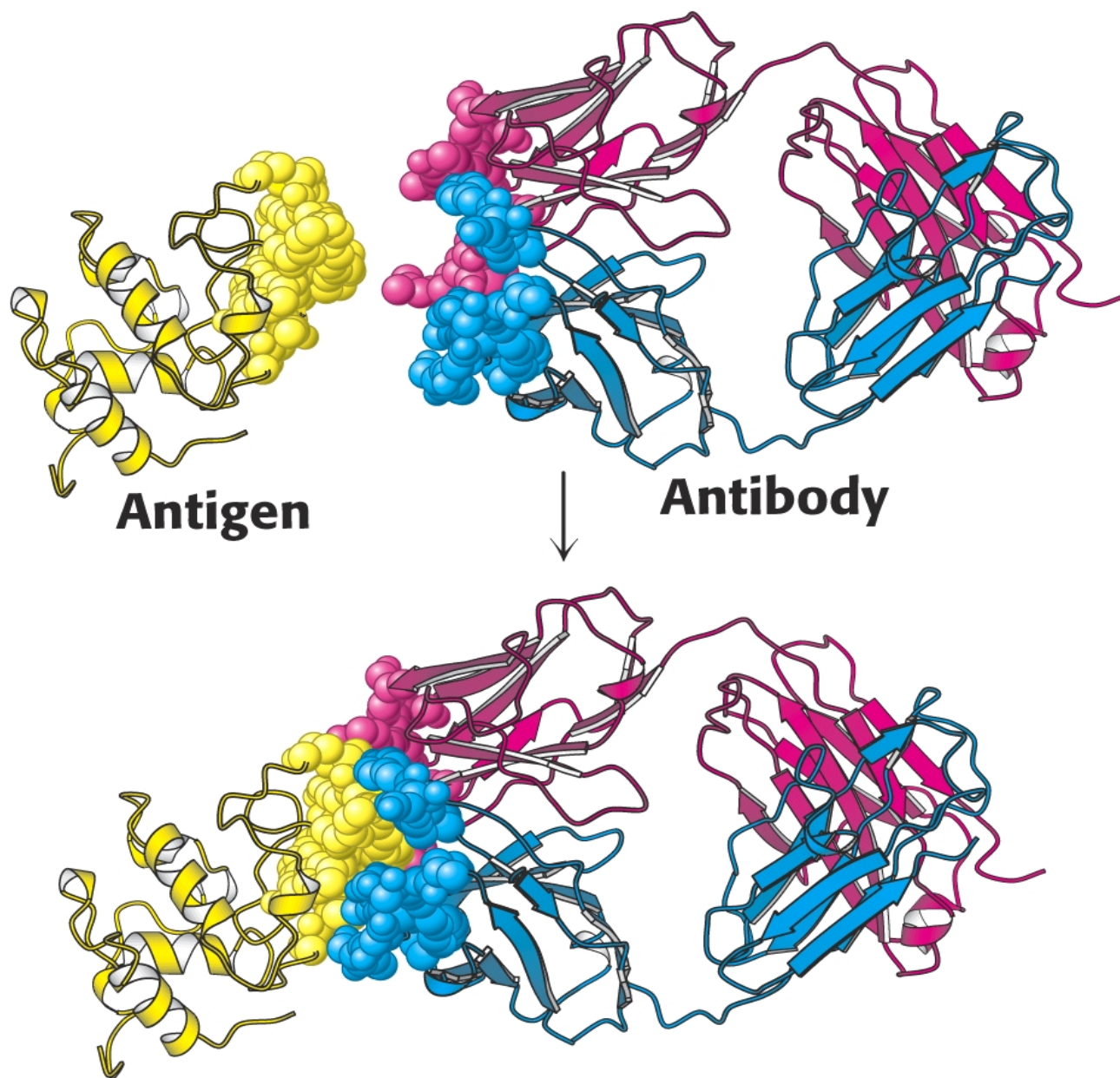
**La lezione del 5 maggio sarà introduttiva al laboratorio
e pertanto la PRESENZA è
FORTEMENTE CONSIGLIATA = OBBLIGATORIA
Sarà fatto l'appello degli iscritti al laboratorio**

**Le esercitazioni avranno luogo
Martedì, Giovedì e Venerdì dalle 14 alle 18 (circa)
Edificio PP1**

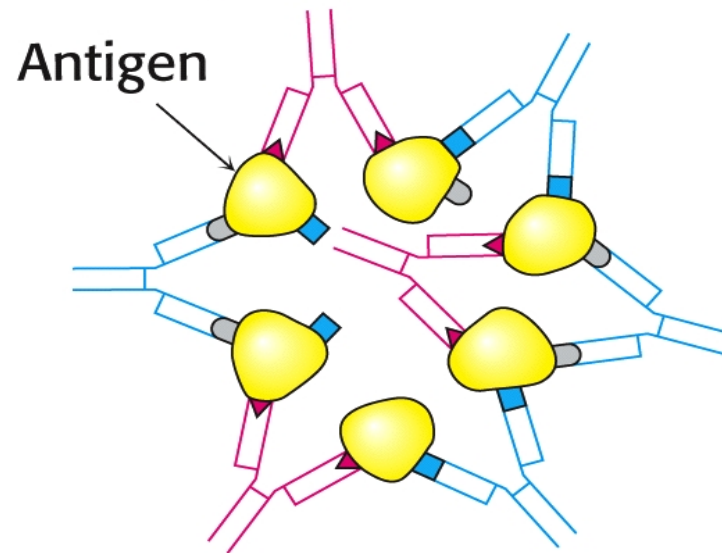
Metodi biochimici che utilizzano anticorpi



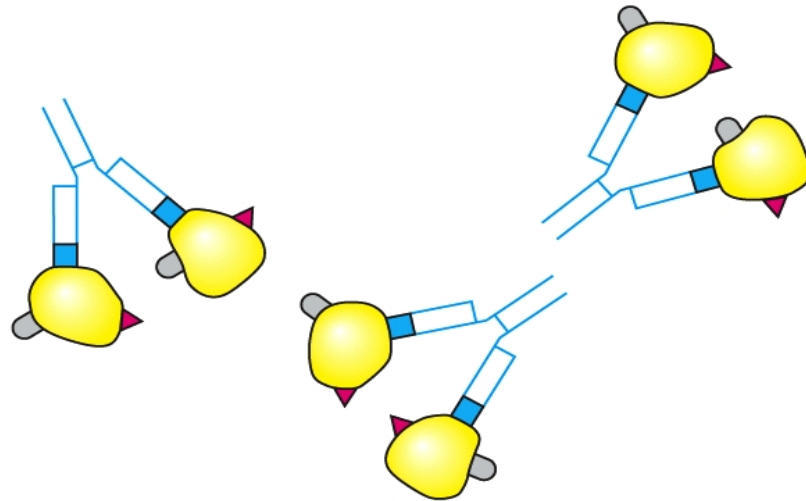




Polyclonal Antibodies

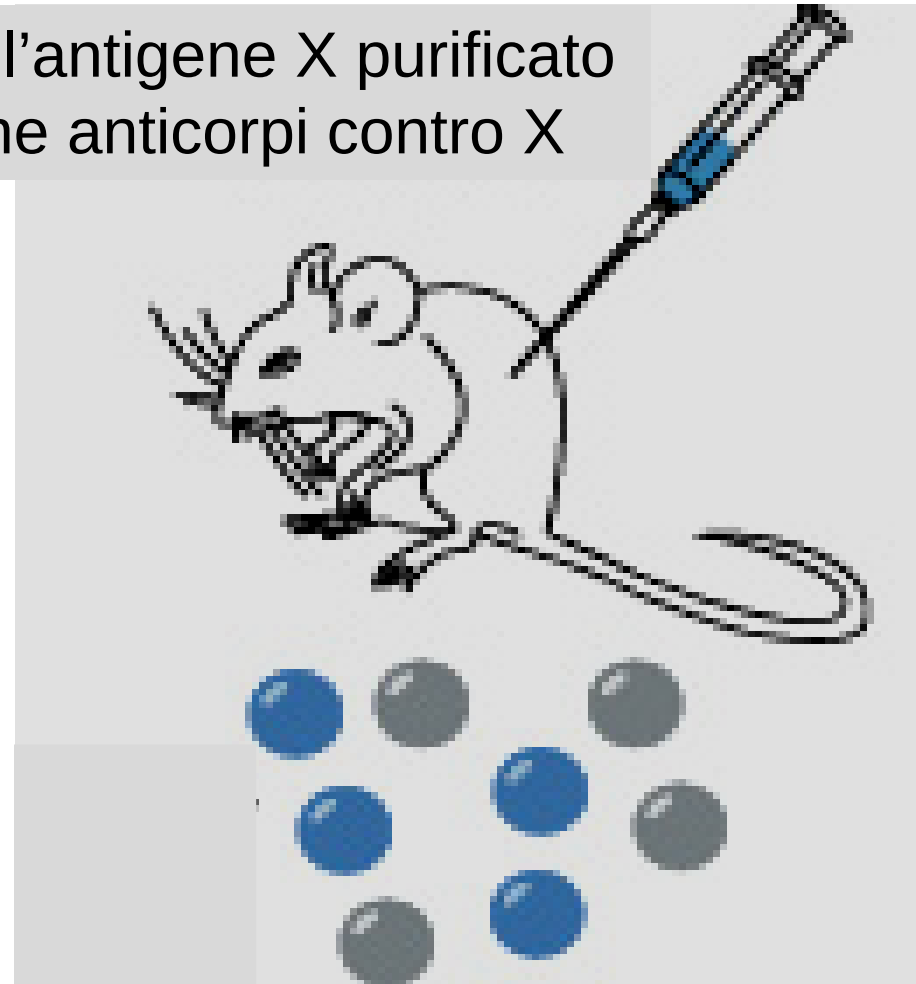


Monoclonal Antibodies

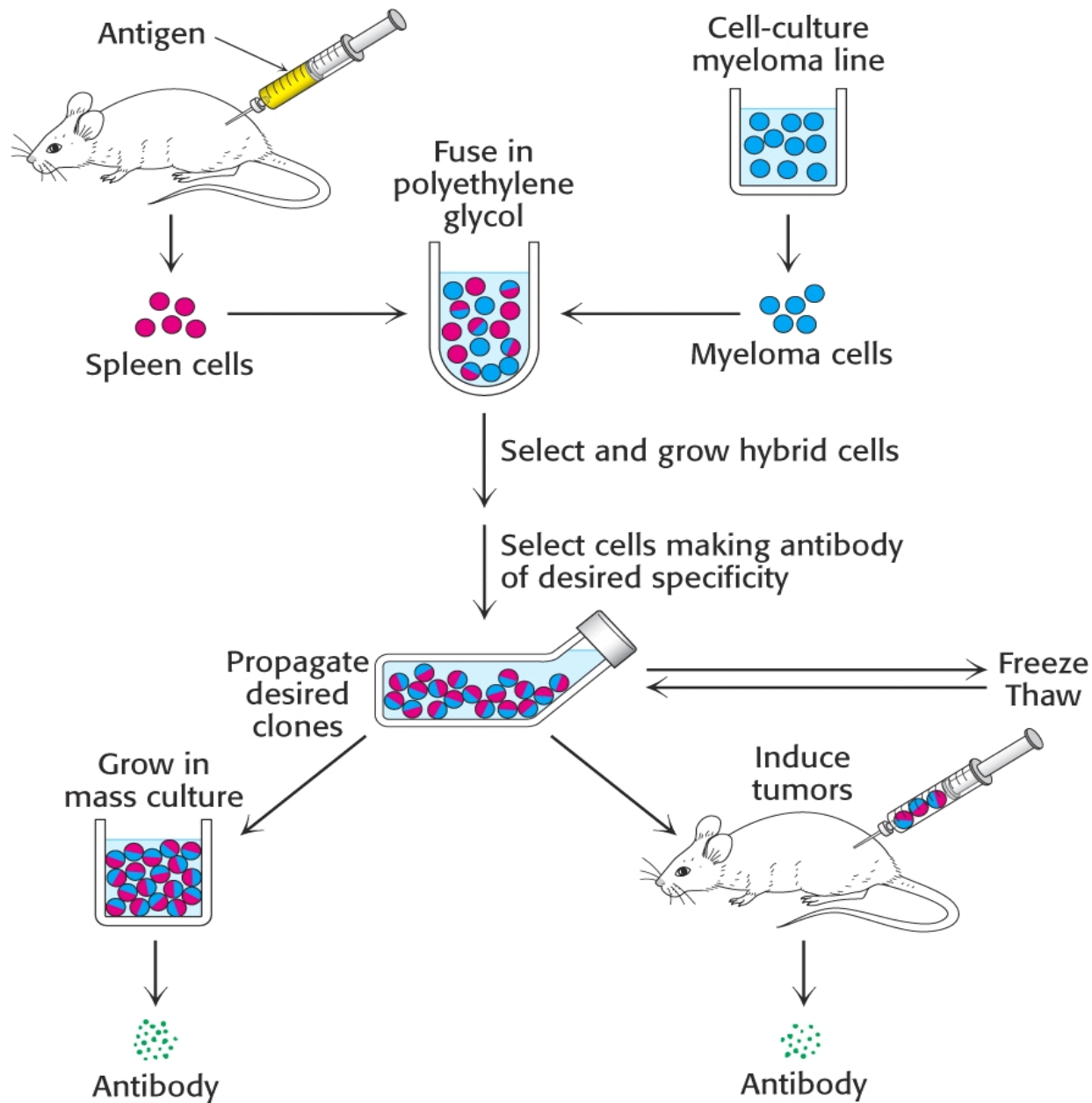


Produzione di anticorpi policlonali

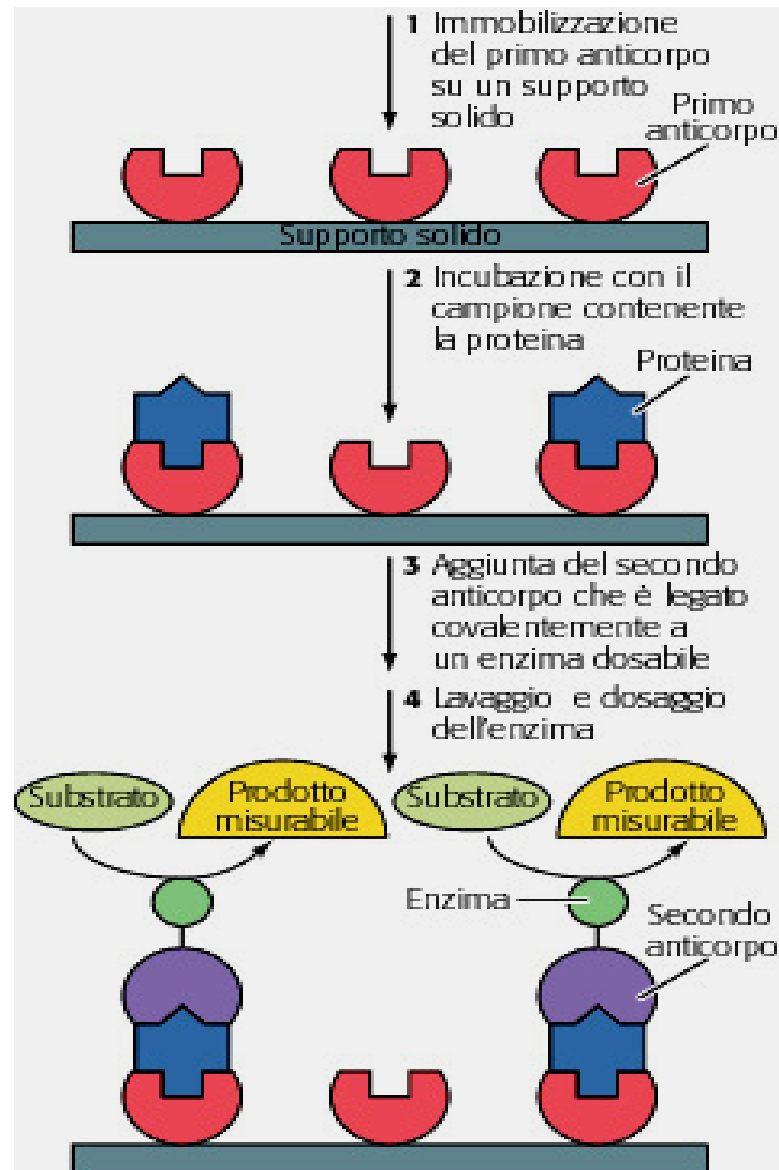
1. Si inietta nel topo (o coniglio) l'antigene X purificato
2. Si preleva il siero, che contiene anticorpi contro X



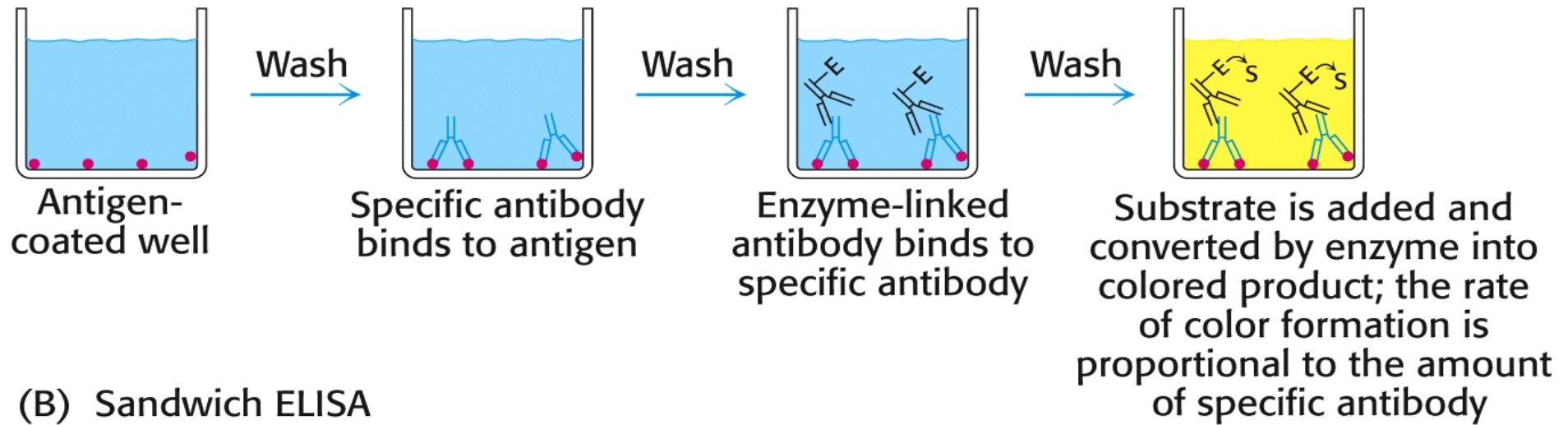
Produzione di anticorpi monoclonali



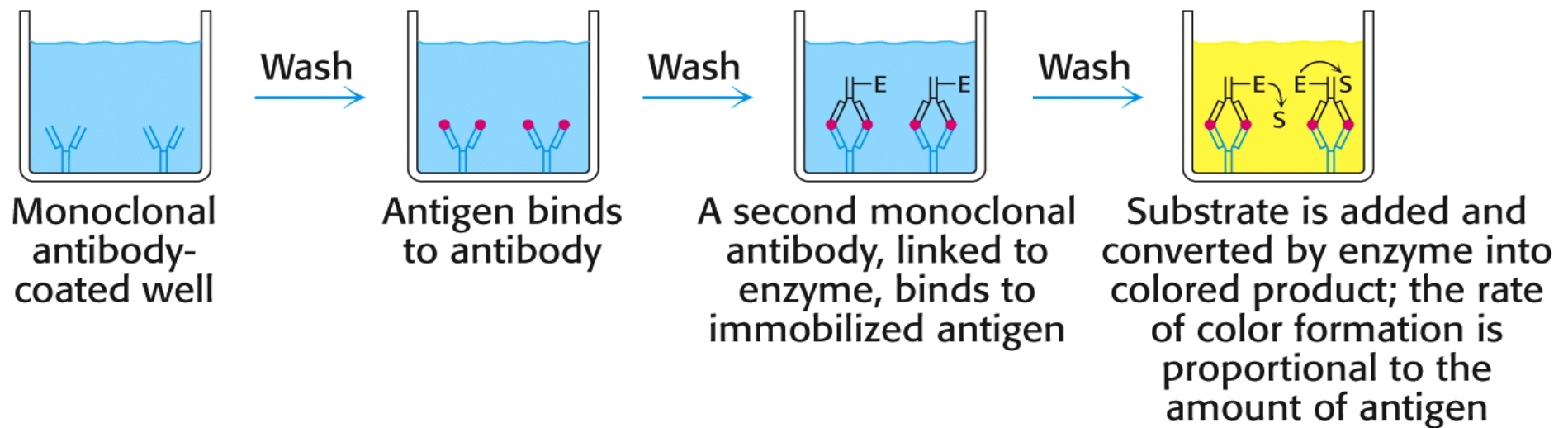
ELISA = Enzyme-Linked ImmunoSorbent Assay



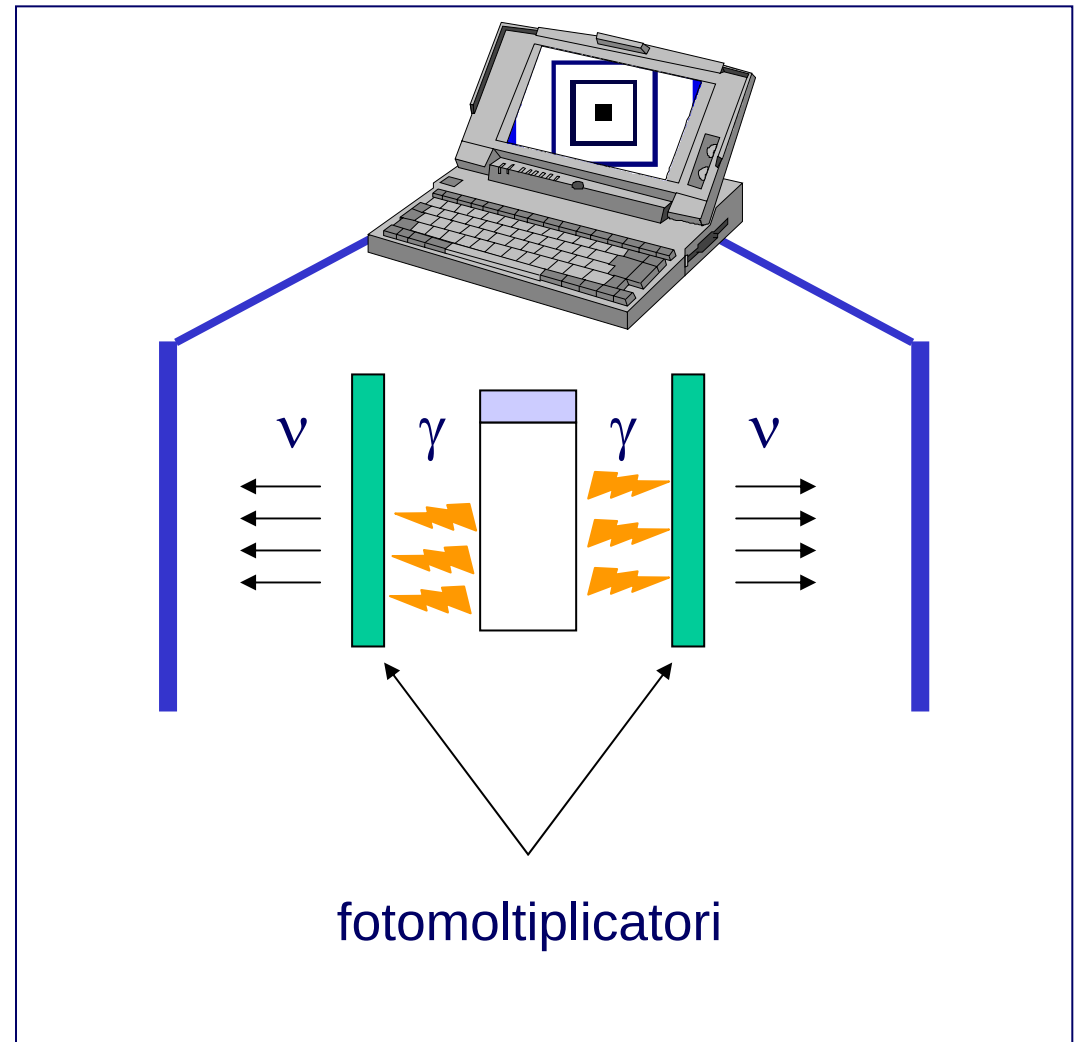
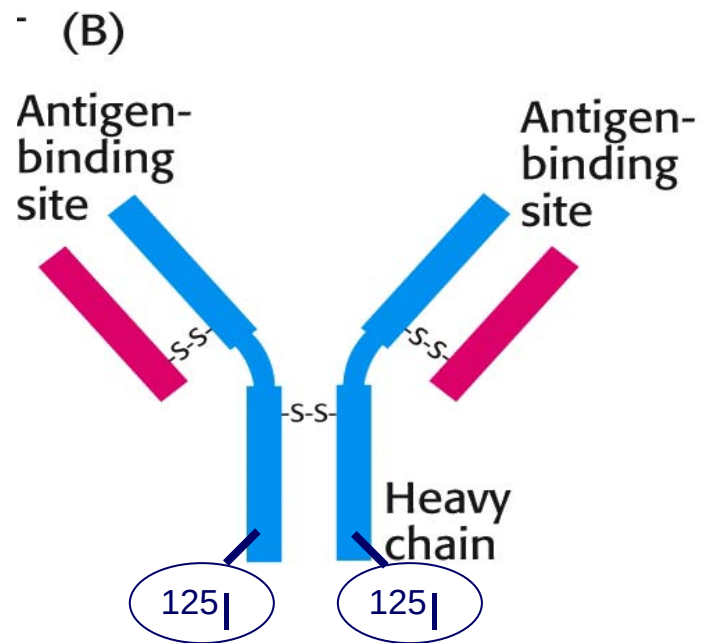
(A) Indirect ELISA



(B) Sandwich ELISA



RIA = Radio-Immuno Assay



LA CITOMETRIA A FLUSSO

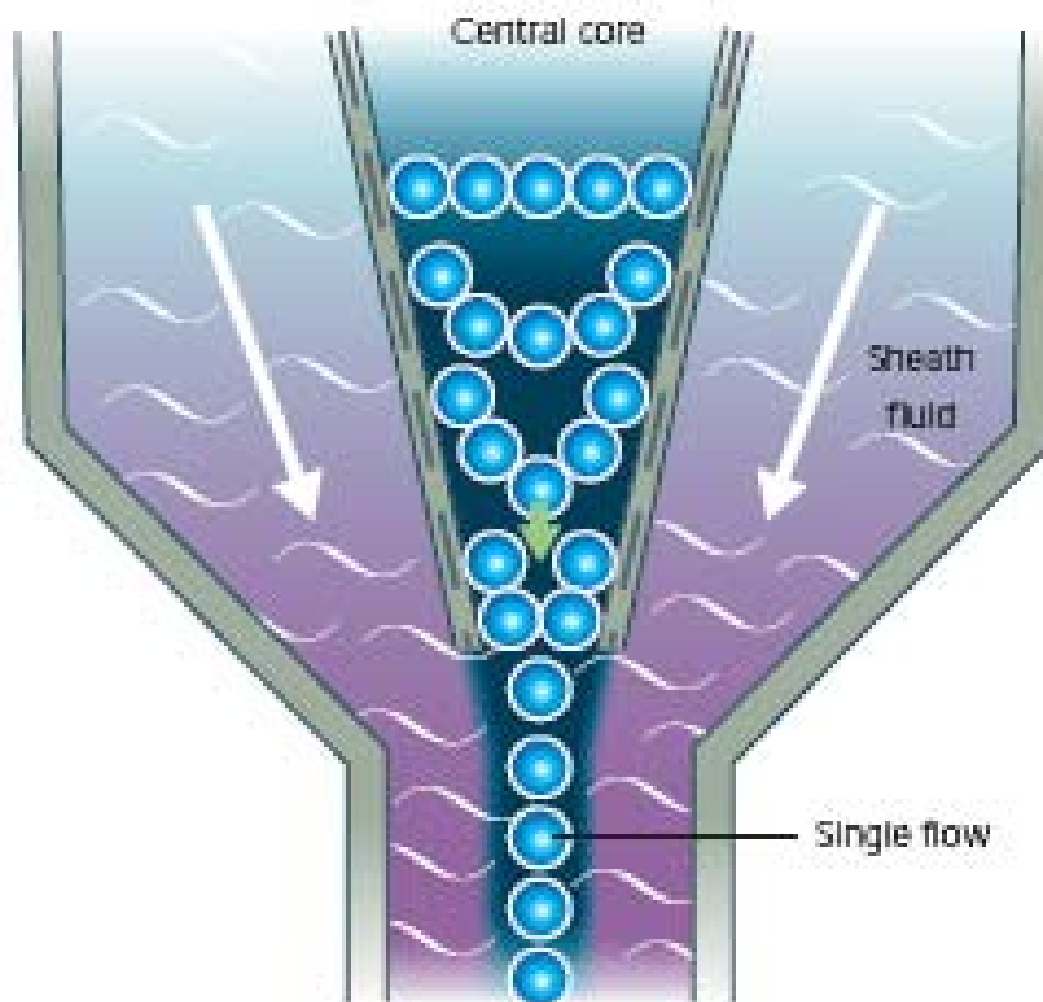
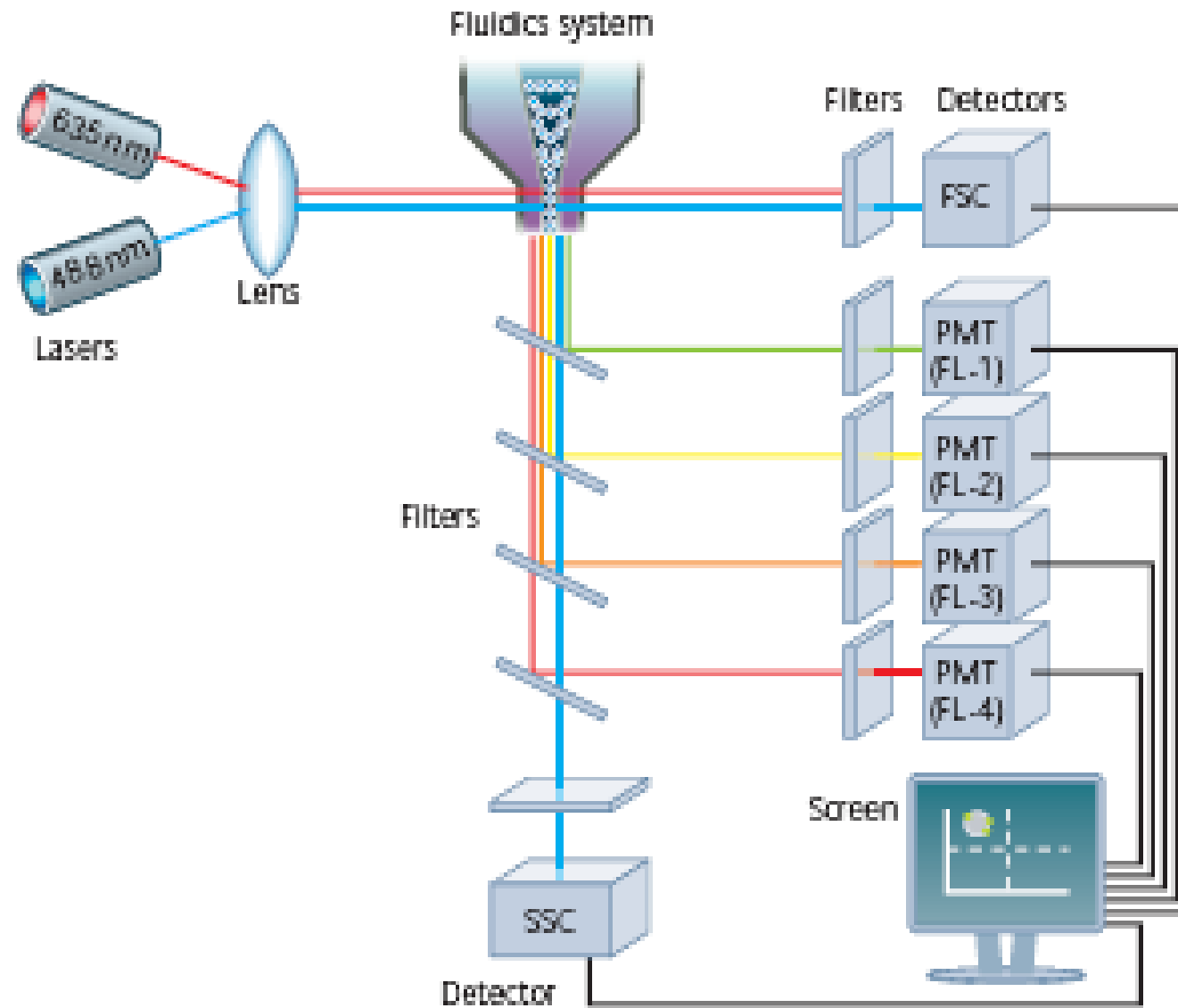


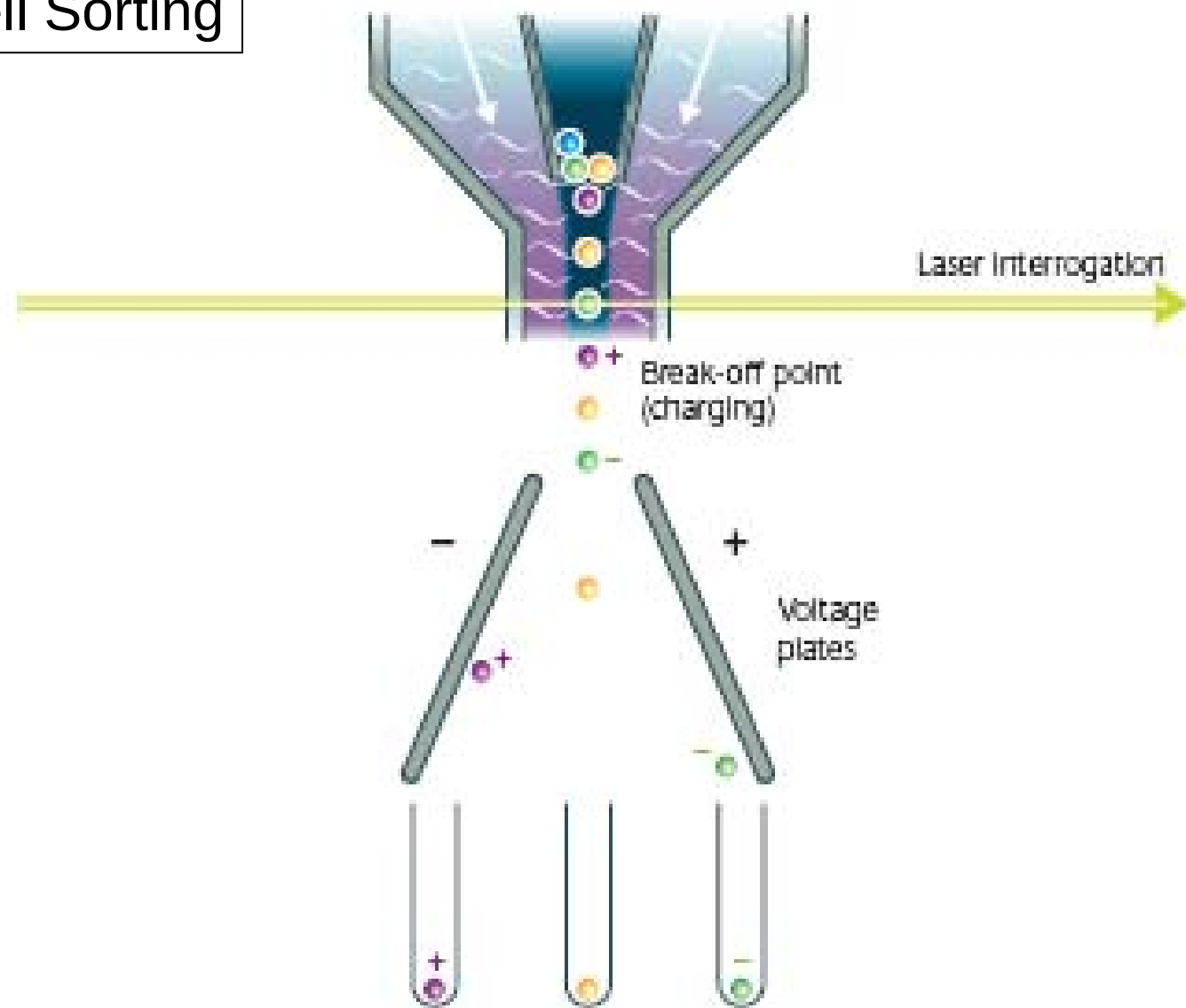
FIGURE 1

Hydrodynamic focusing produces a single stream of particles

Schema di un citoflurimetro a flusso



Electrostatic Cell Sorting



Electrostatic flow sorting

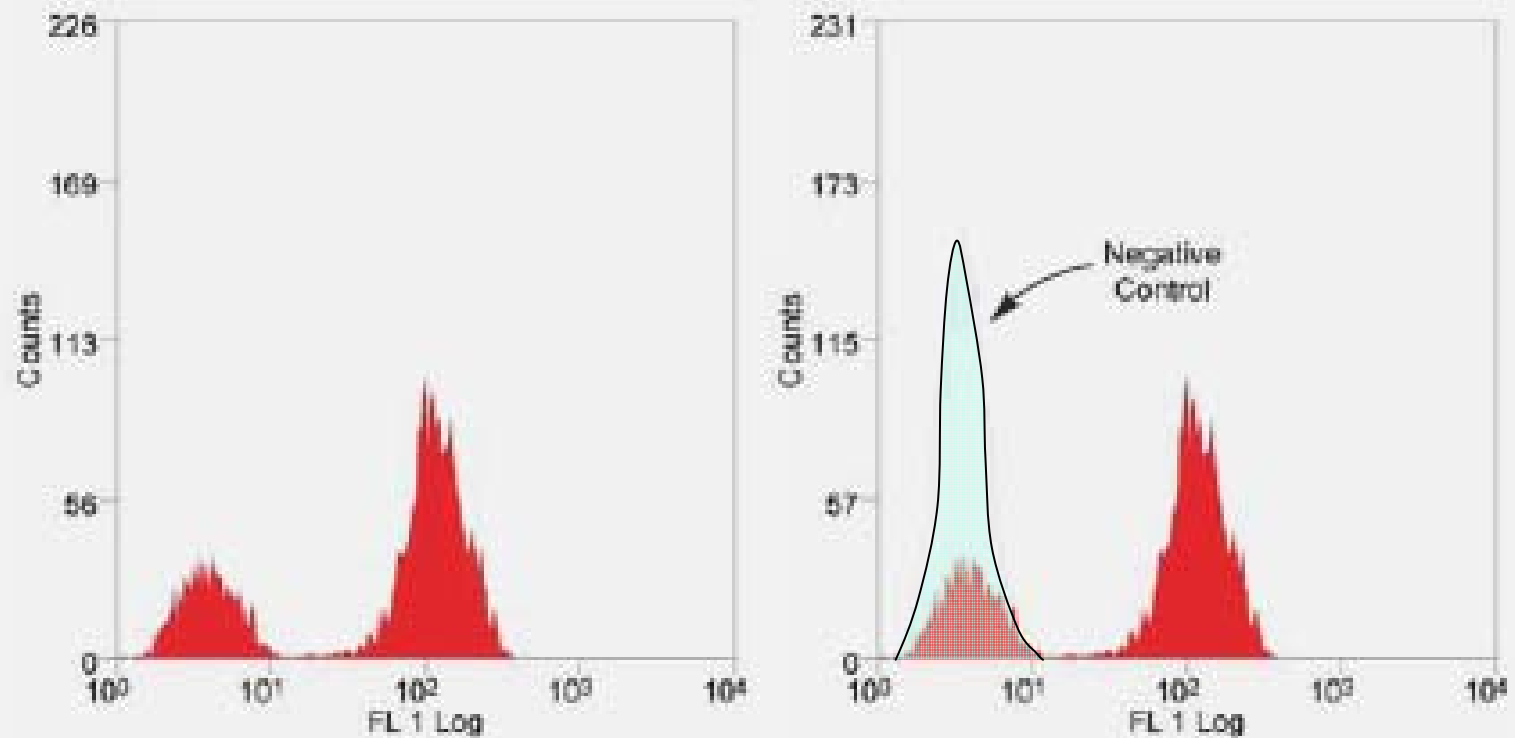
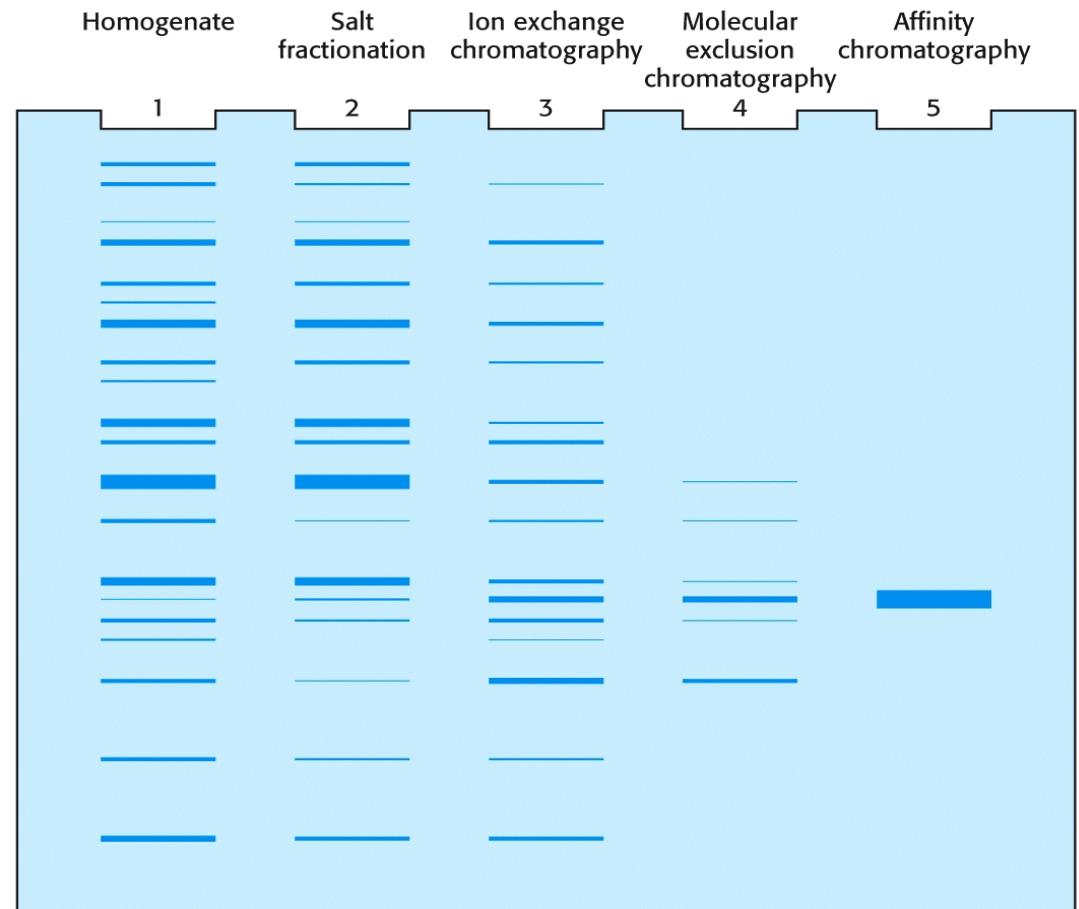


FIGURE 12 Which is the positive dataset? LEFT, Using rat anti-mouse F4/80 conjugated to FITC to stain mouse peritoneal macrophages produces two peaks. RIGHT, By running an appropriate isotype control (rat IgG2b negative control conjugated to FITC) and overlaying its image on the histogram (blue outline) the positive dataset is identified as the taller red peak on the right.

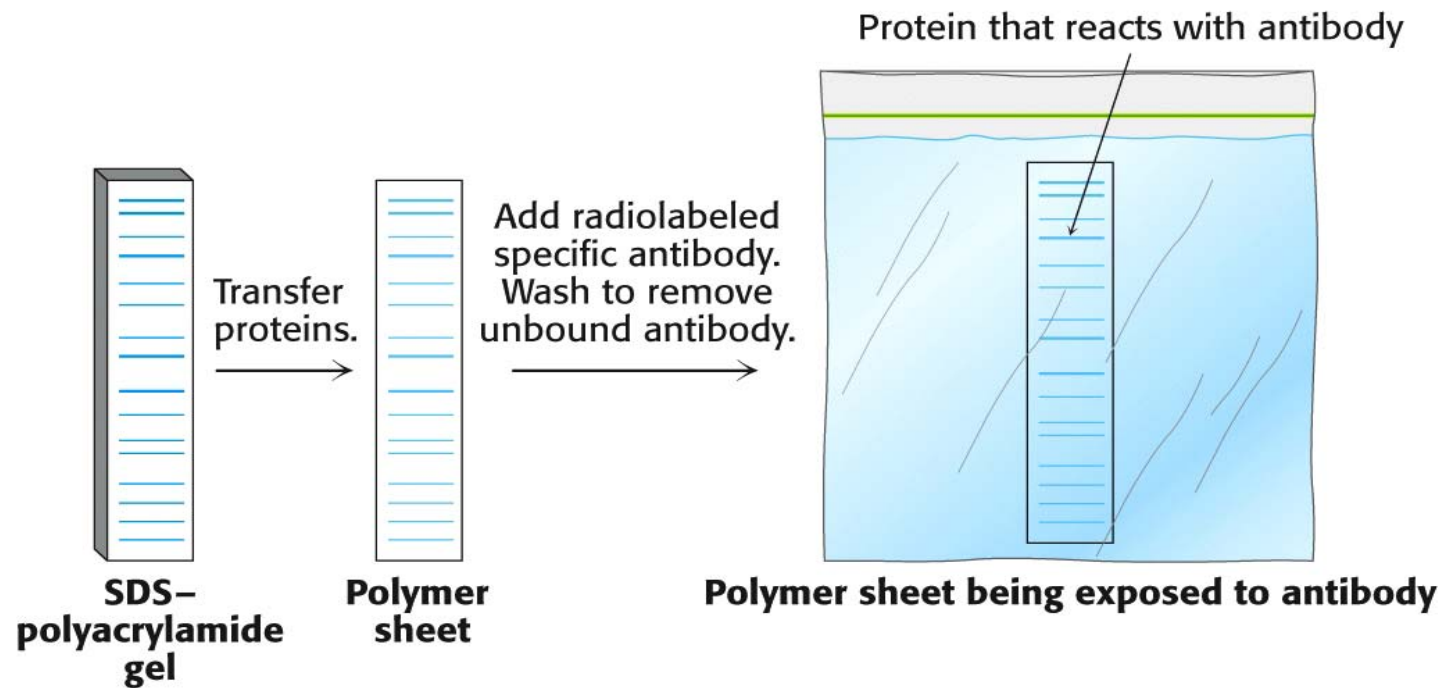
Western blot.1

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



Western blot. 2

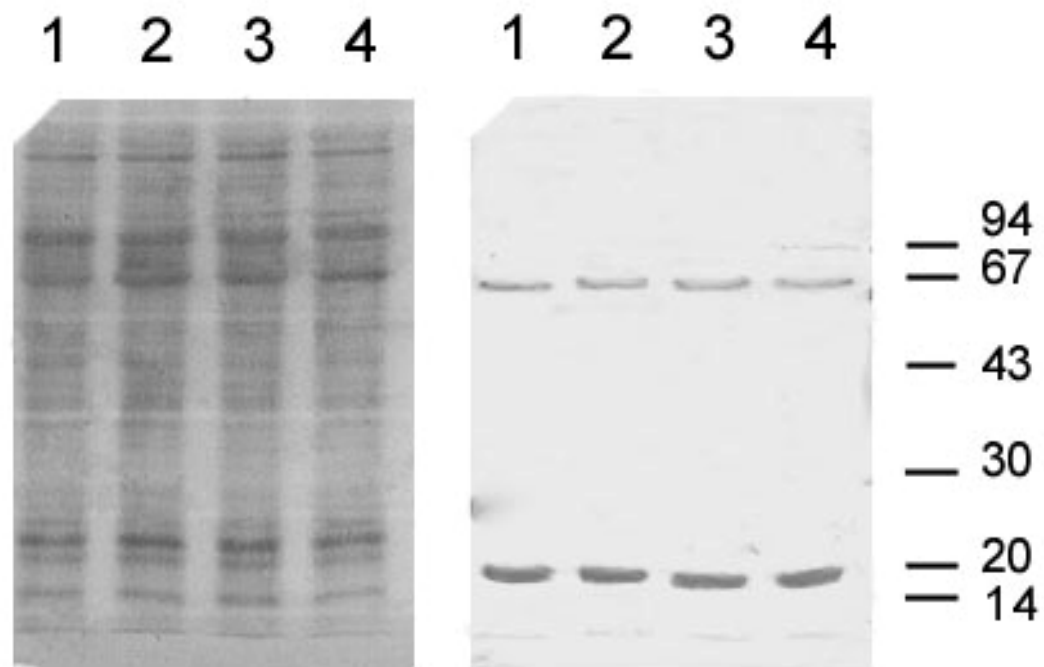
3. Trasferimento delle proteine dal gel ad una membrana (nitrocellulosa o PVC)
4. Saturazione di siti di legame non specifici (BSO)
5. Incubazione con anticorpo primario



Western blot. 3

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

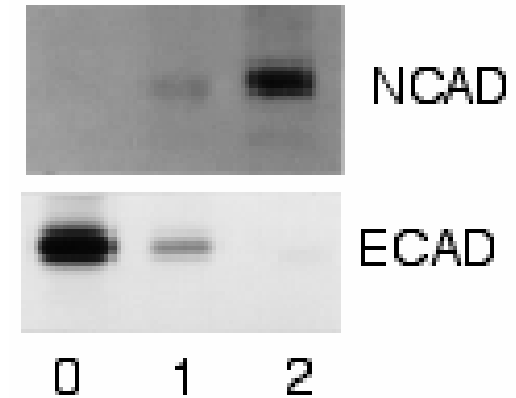
7. Detection



7. Detection

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

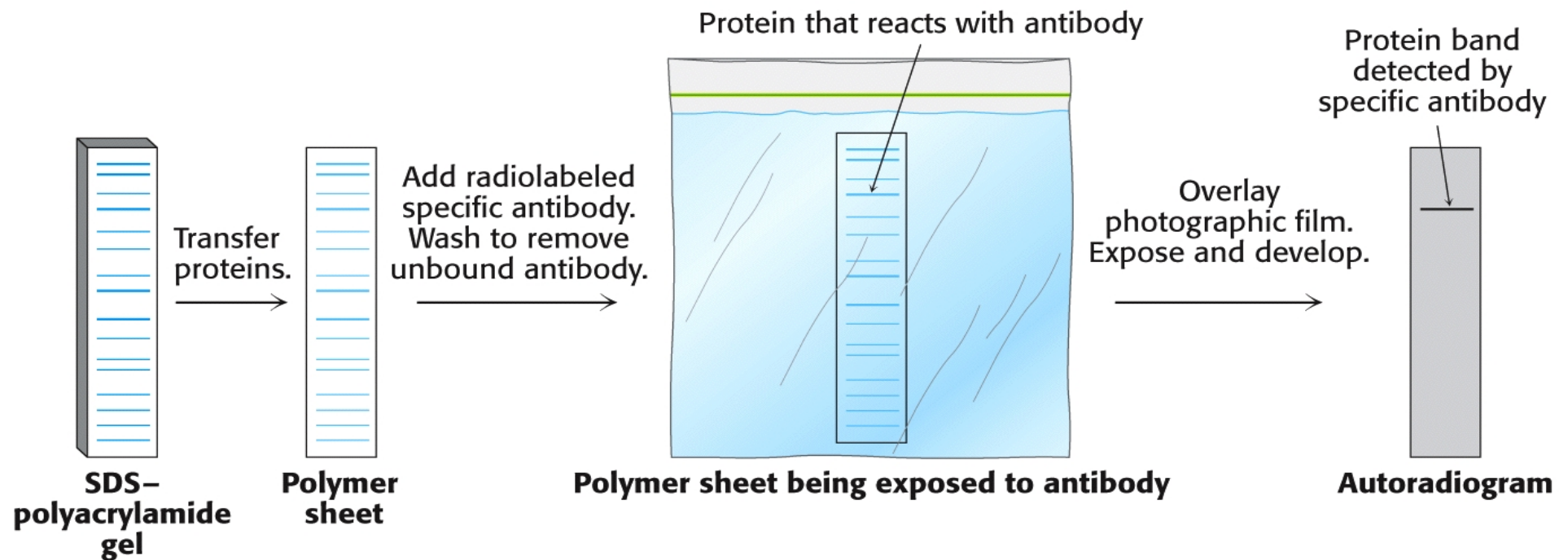
- Colorata
- Luminescente
- Fluorescente



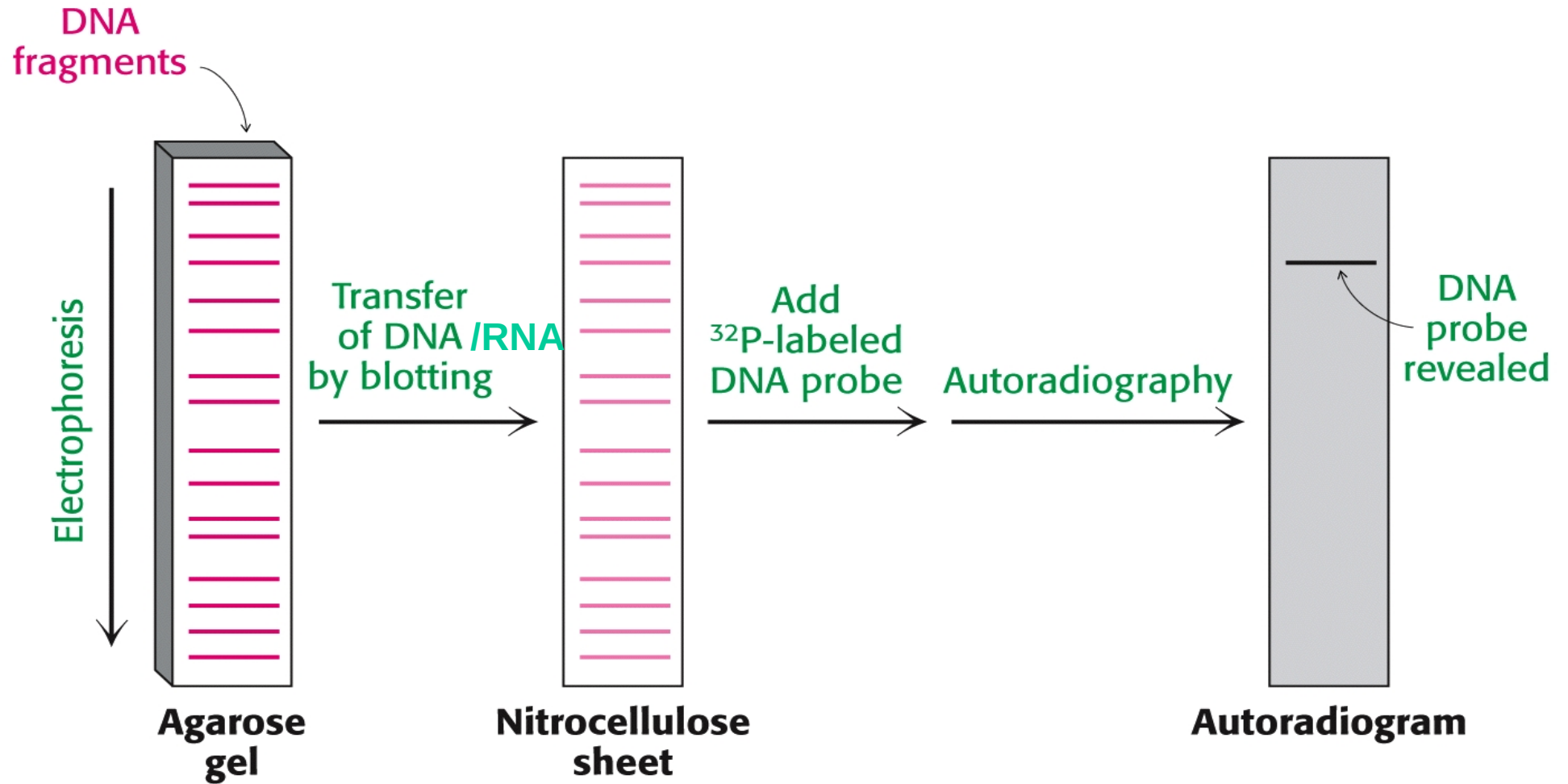
Sistema di visualizzazione ed analisi
(densitometria)

7. Detection

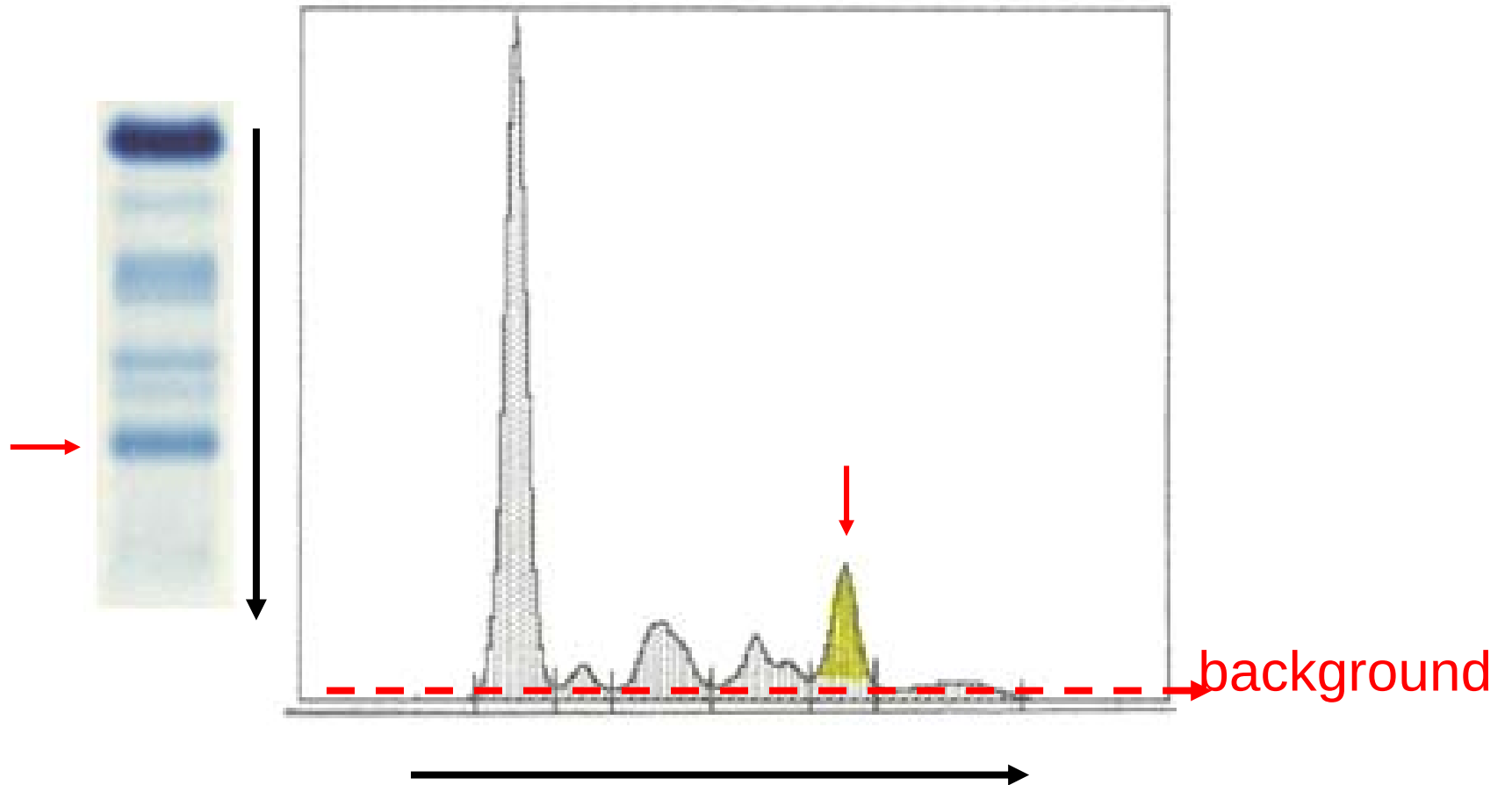
2. L'anticorpo secondario è marcato con un isotopo radioattivo



Southern/Northern blot.1



Quantitation



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

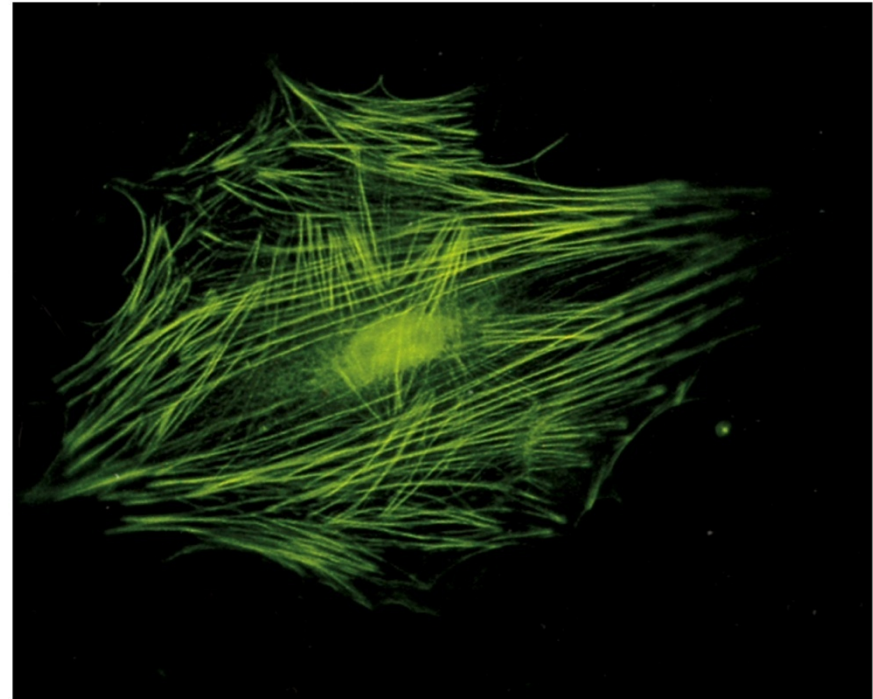


Altre molecole
non affini al ligando



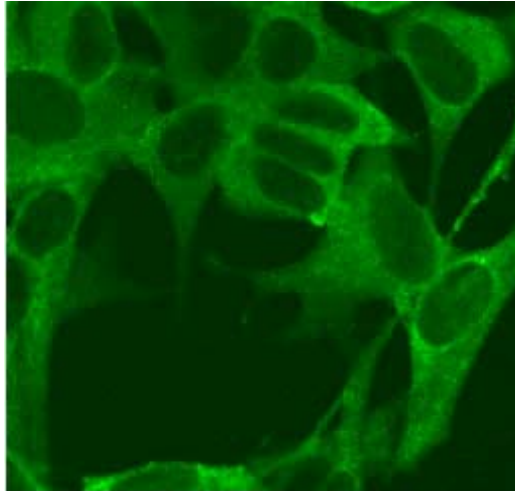
Immunoistochimica

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

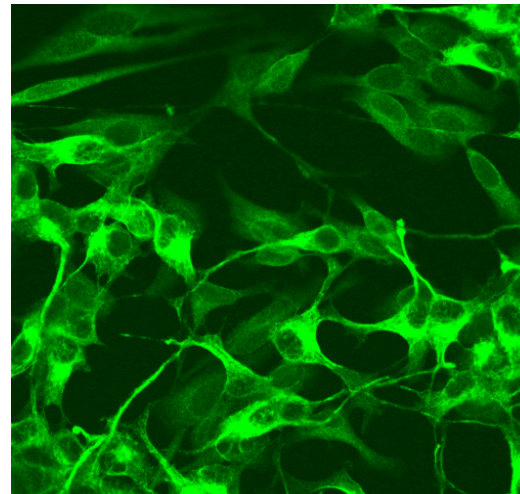
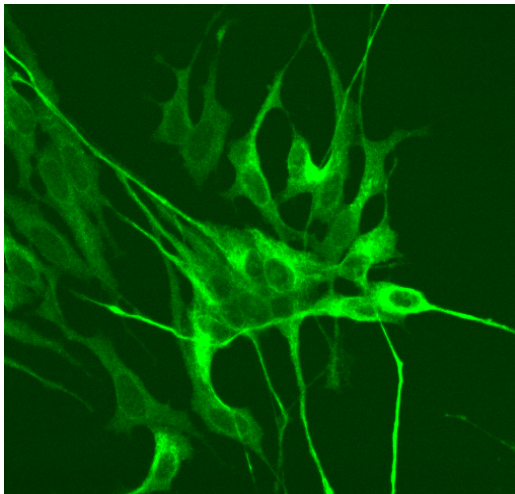
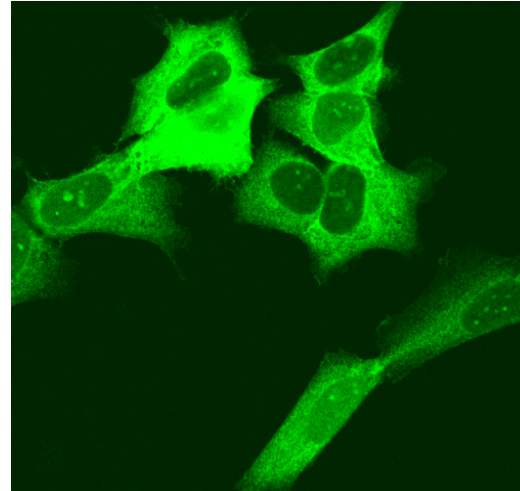


L'immunoistochimica è semi-quantitativa

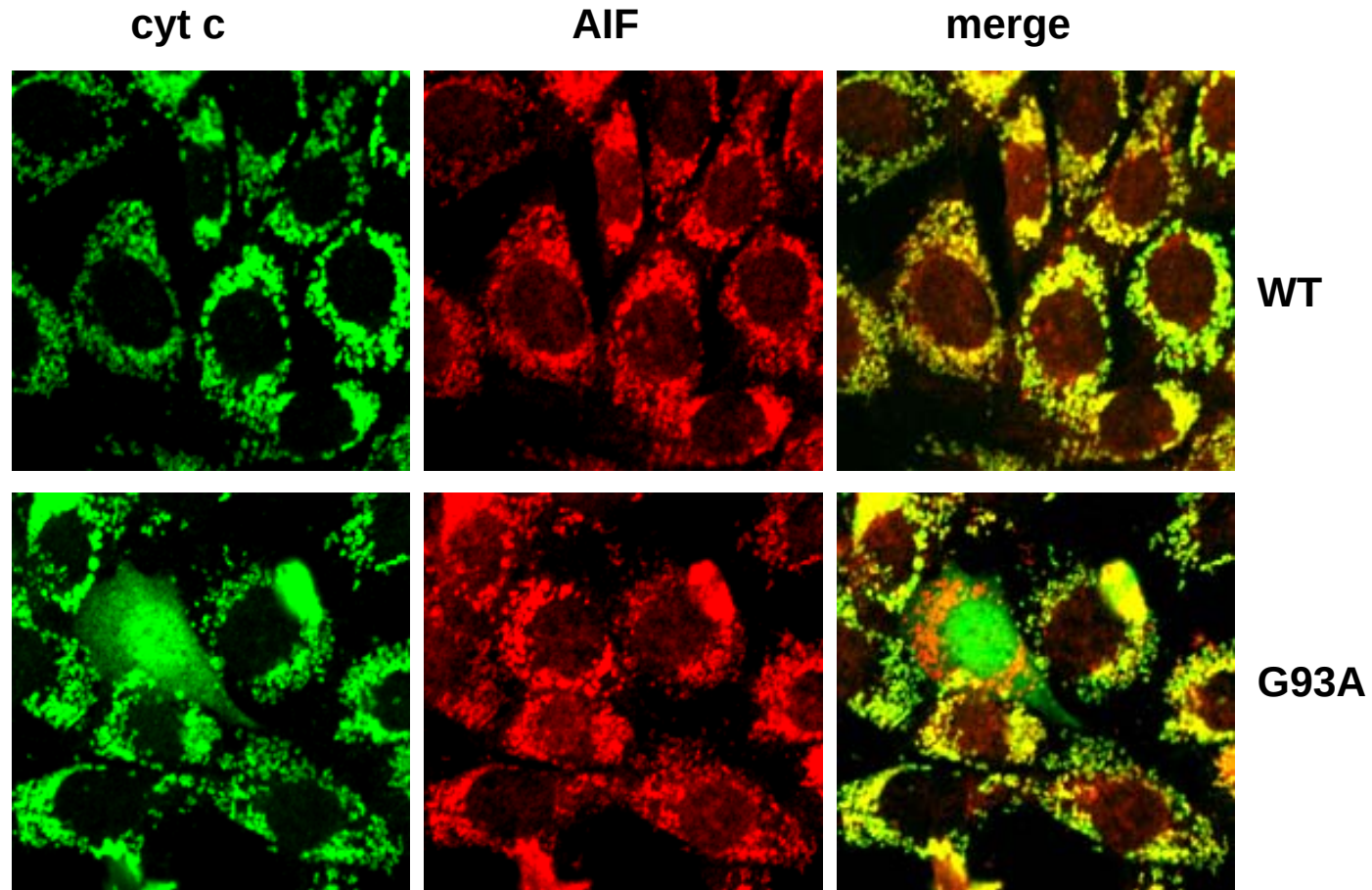
SH



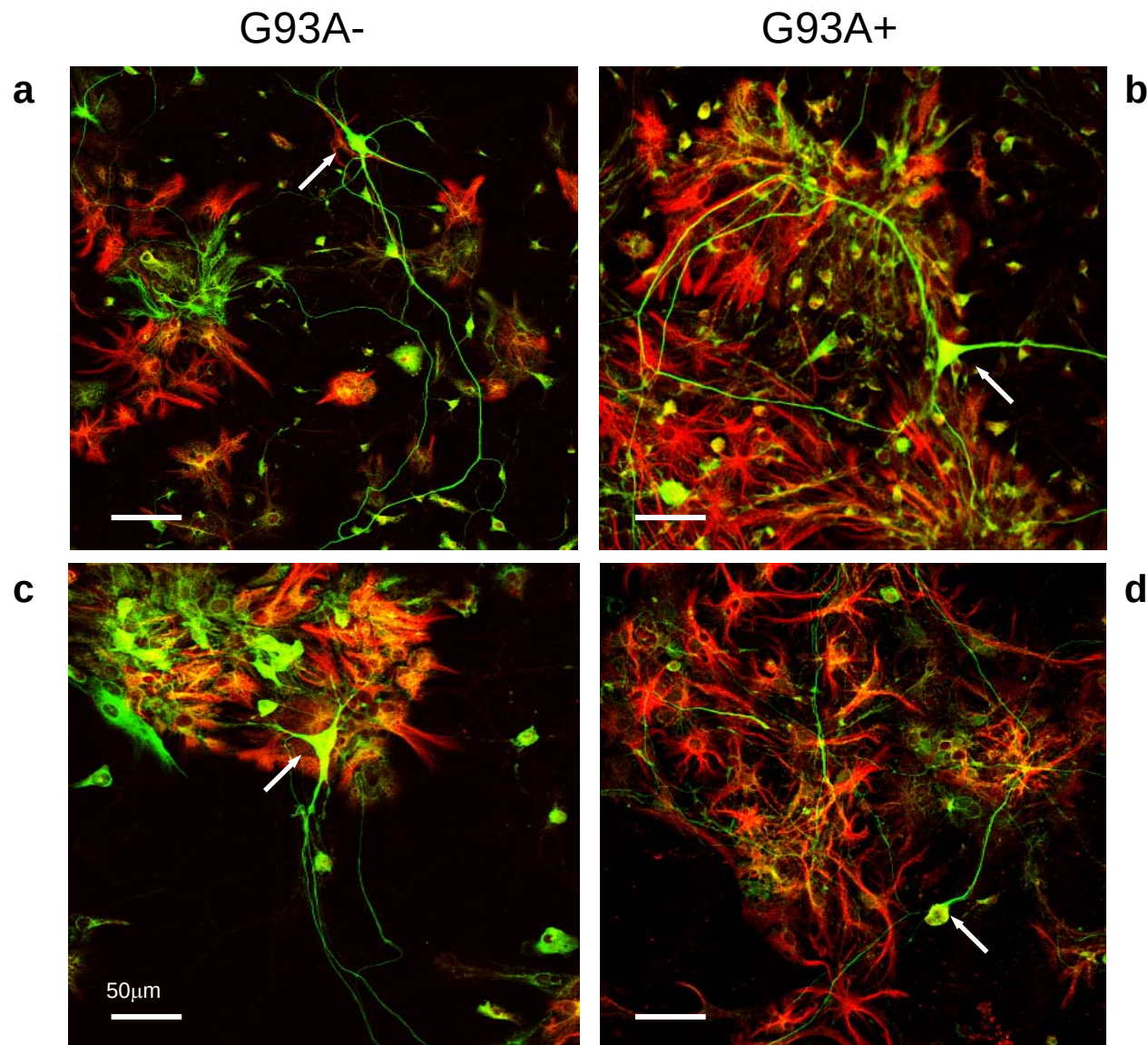
WT



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)

