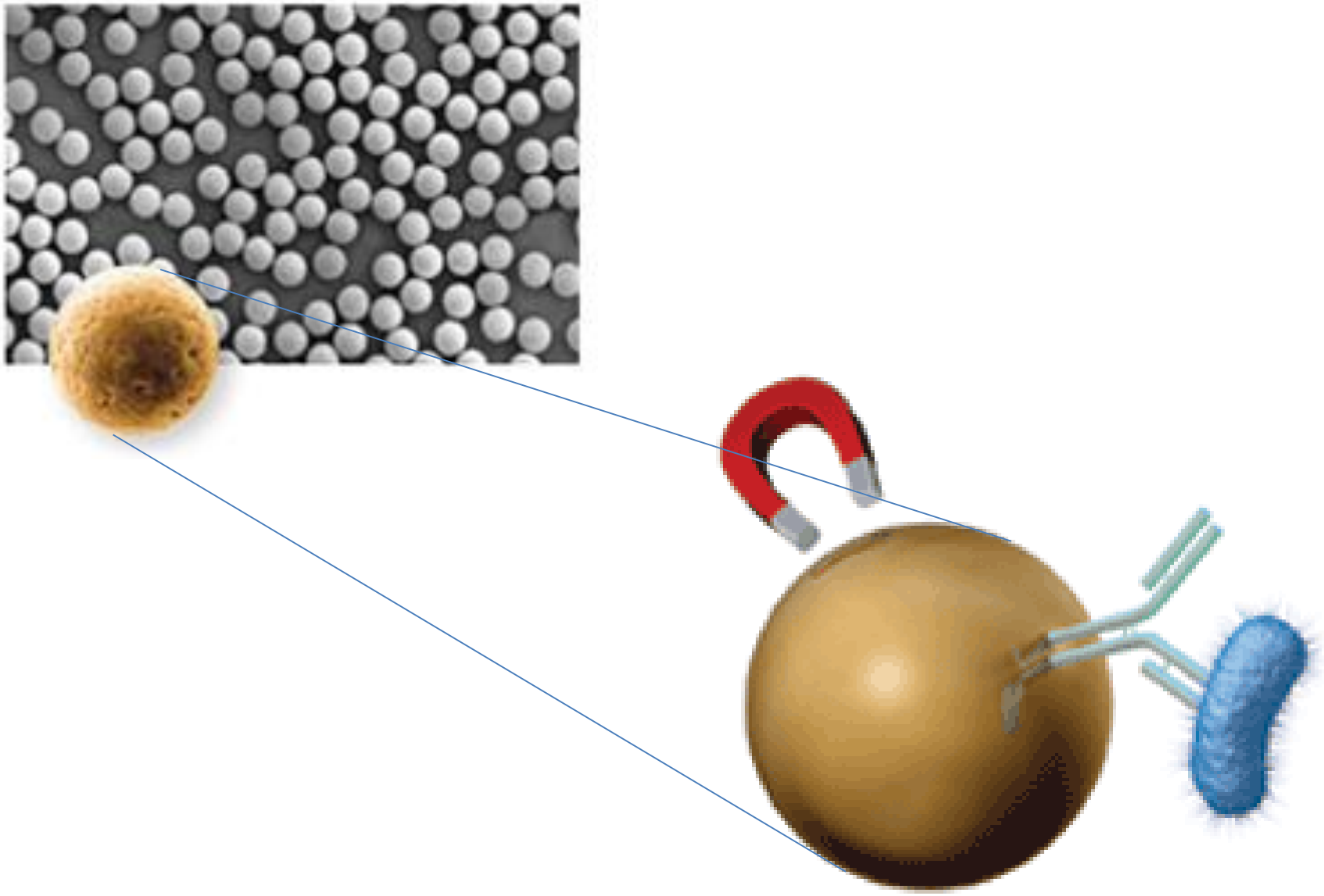
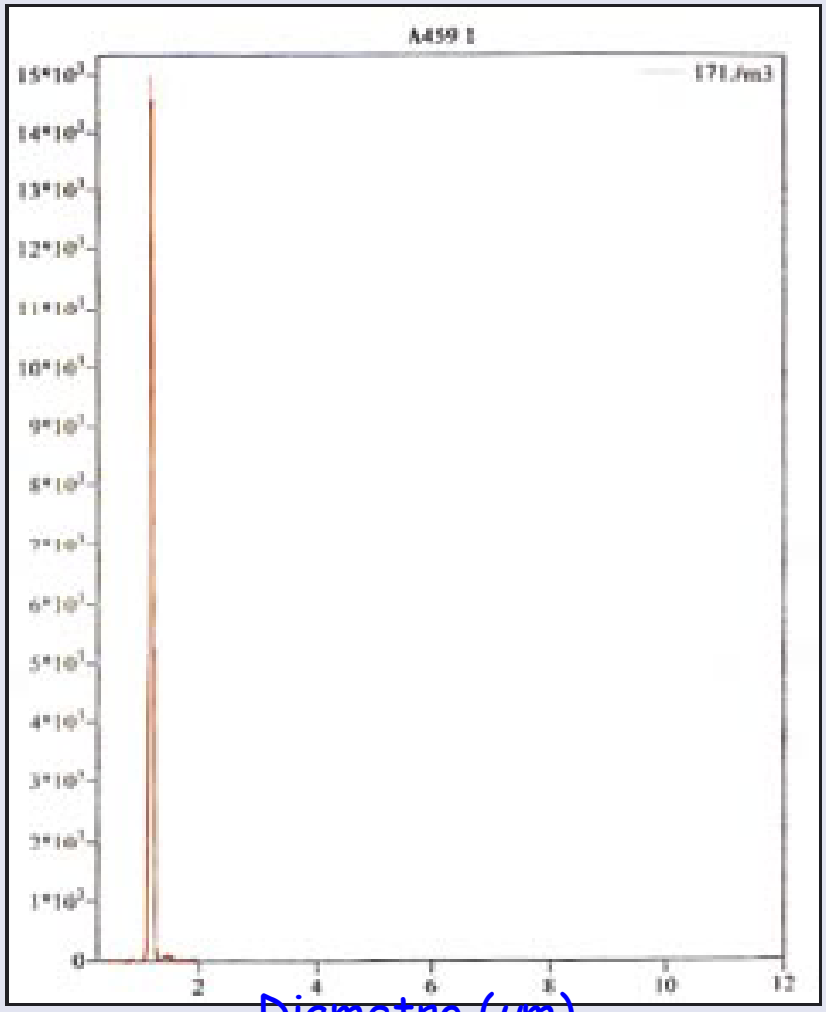
Dynabeads

Invitrogen™
life technologies

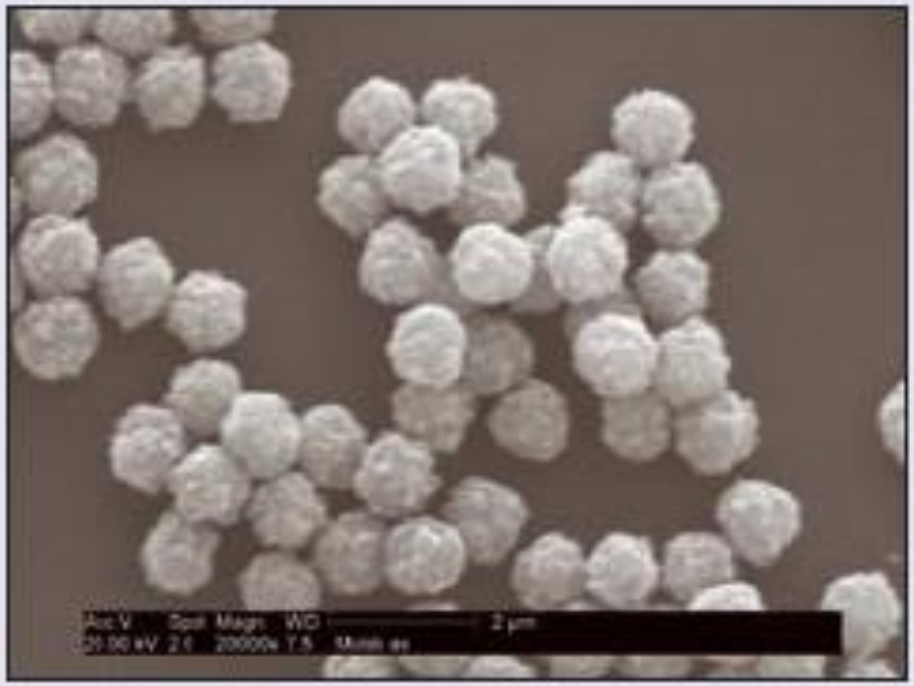


Isolamento di molecole e cellule

Biglie magnetiche



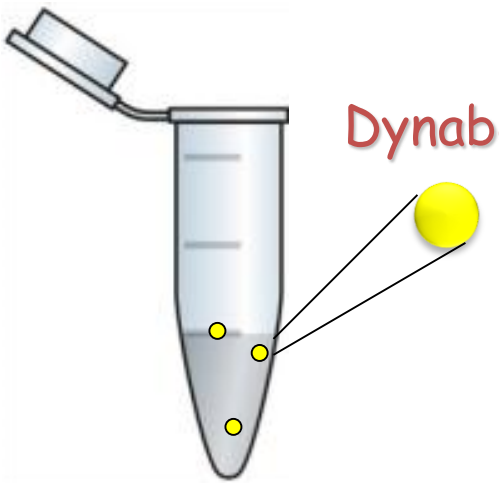
Dynabeads® MyOne™ size distribution.



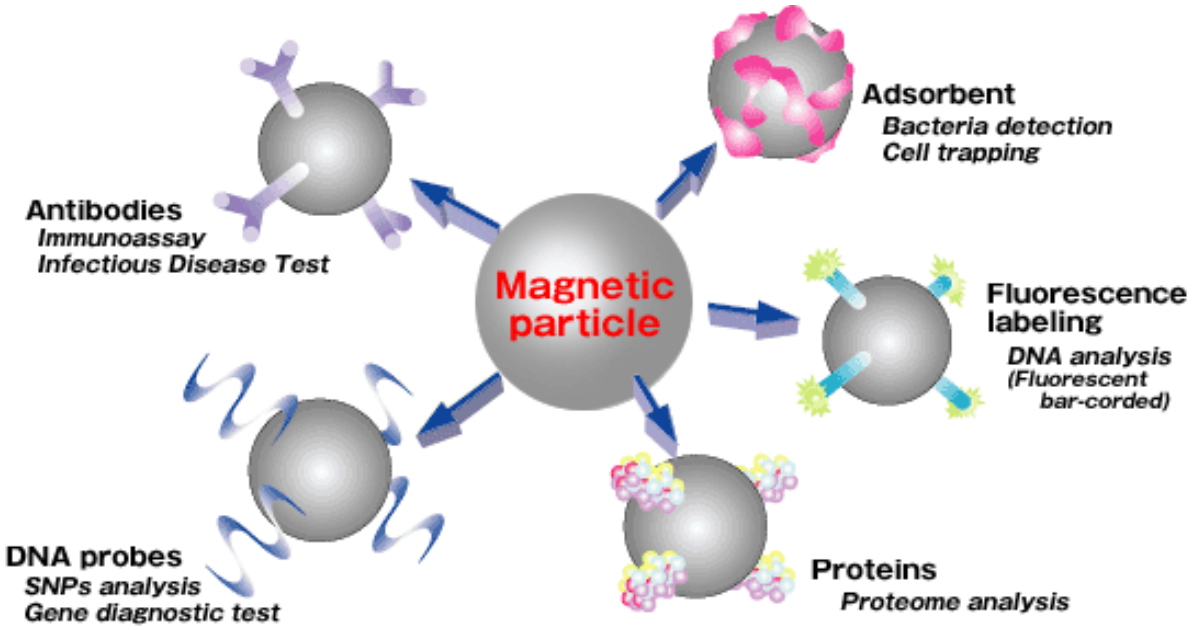
Dynabeads® MyOne™ (SEM picture).

Dynabeads® are uniform, monodisperse and superparamagnetic particles. Constant iron content give a strong and even pull to the magnet.

Isolamento di molecole



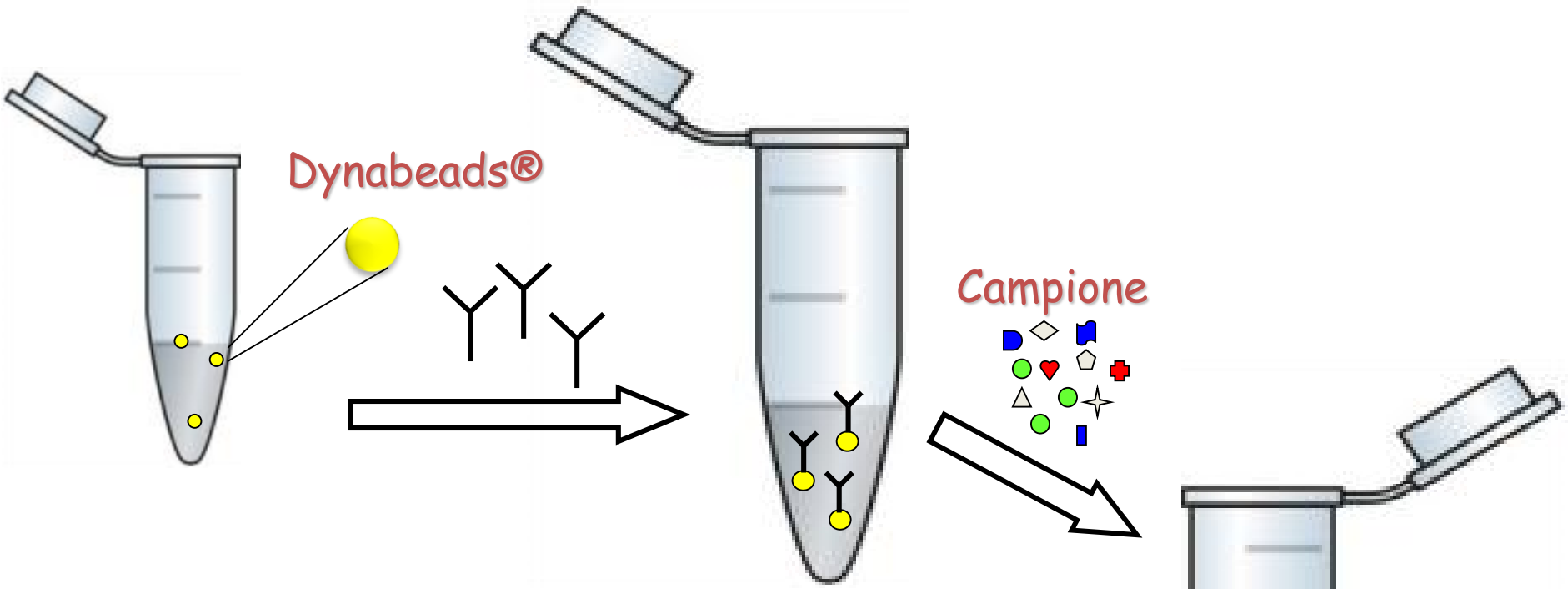
Dynabeads®



Biglie magnetiche con differenti gruppi chimici sulla superficie, adatti per legarvi diverse molecole.

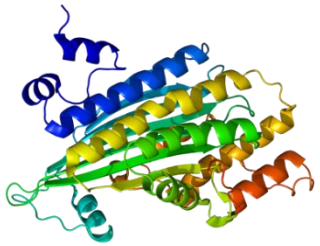
Riutilizzabili.

Isolamento di molecole



Biglie magnetiche con differenti gruppi chimici sulla superficie, adatti per legarvi diverse molecole.

Riutilizzabili.



Isolamento di proteine

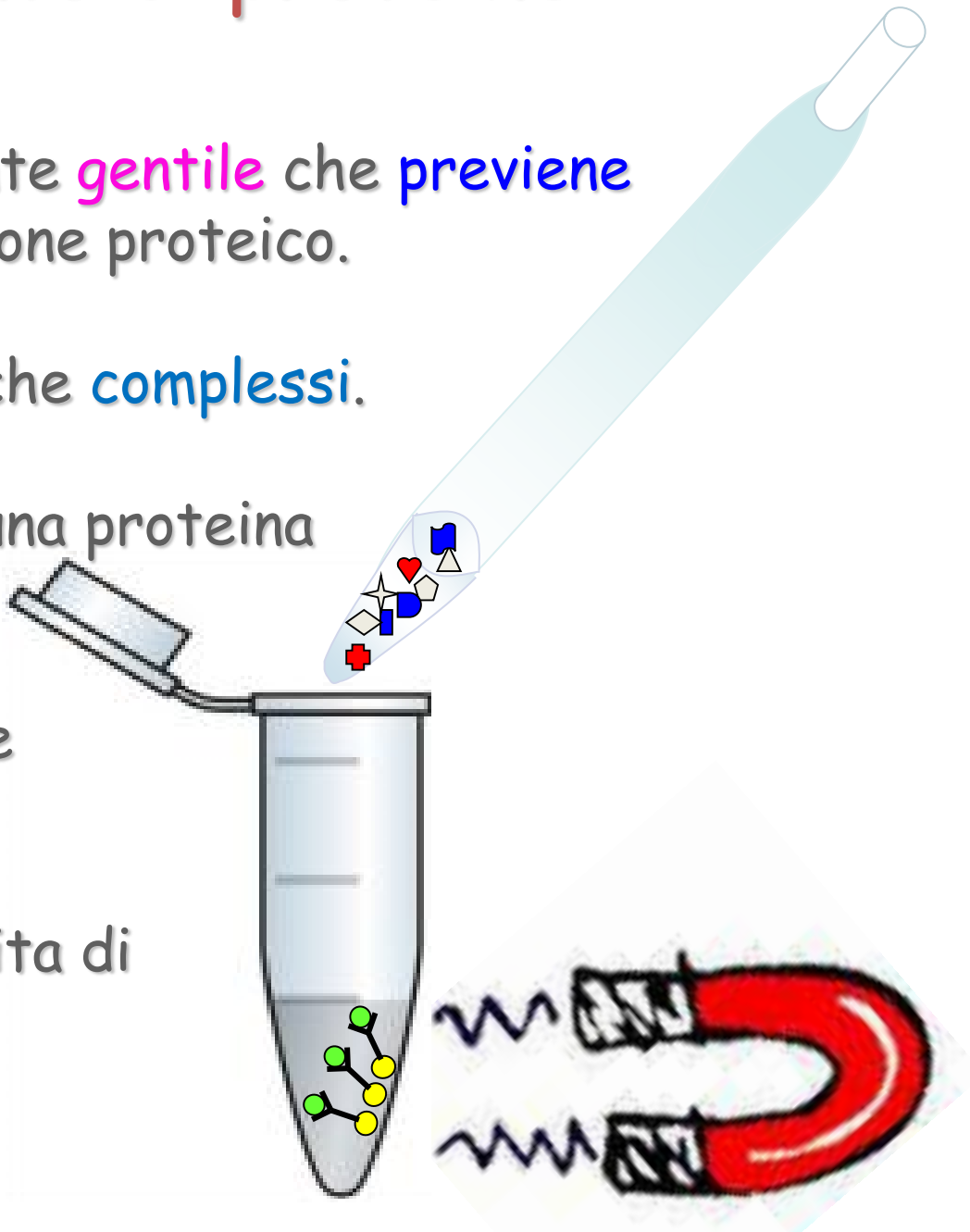
- Separazione estremamente gentile che previene la denaturazione del campione proteico.

- Permette di separare anche complessi.

- Usata per deplezione di una proteina da un campione.

- Utilizzabili per migliorare le immunoprecipitazioni.

- Poche fasi = minima perdita di campione.



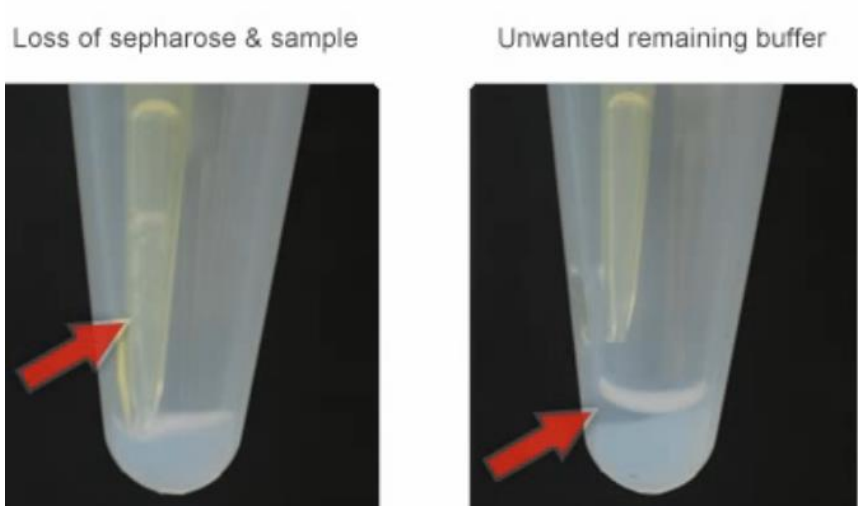
Altri vantaggi nell'isolamento di proteine

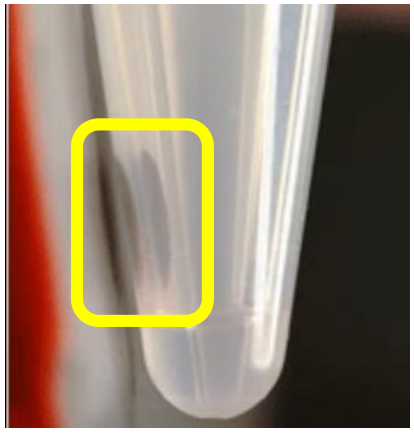
Facilità nei lavaggi, sostituzioni di buffer ed eluizioni.

Background ridottissimo, che evita la necessità del blocking.

Velocità di utilizzo.

Minima perdita di materiale (campione e biglie).





Magneti

16 provette da 1.5-2 mL
Volume di lavoro: 10-2,000 µL

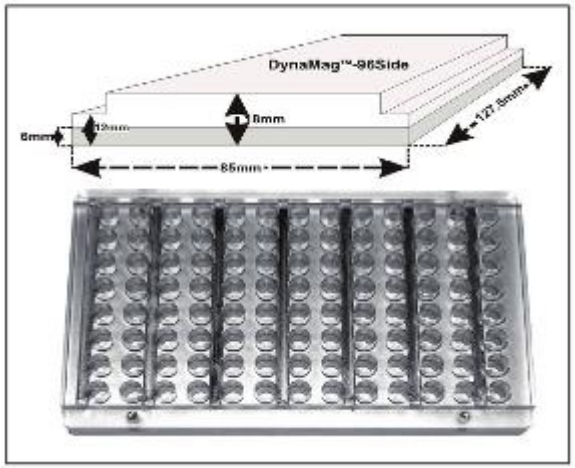
524 euro

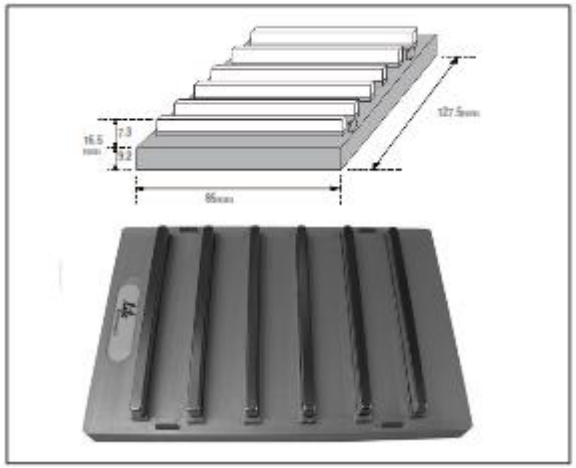
8 provette da 1-5 mL
Volume di lavoro: 10-5,000 µL

625 euro

96 pozzetti da 200 uL
Volume di lavoro: 5-200 µL

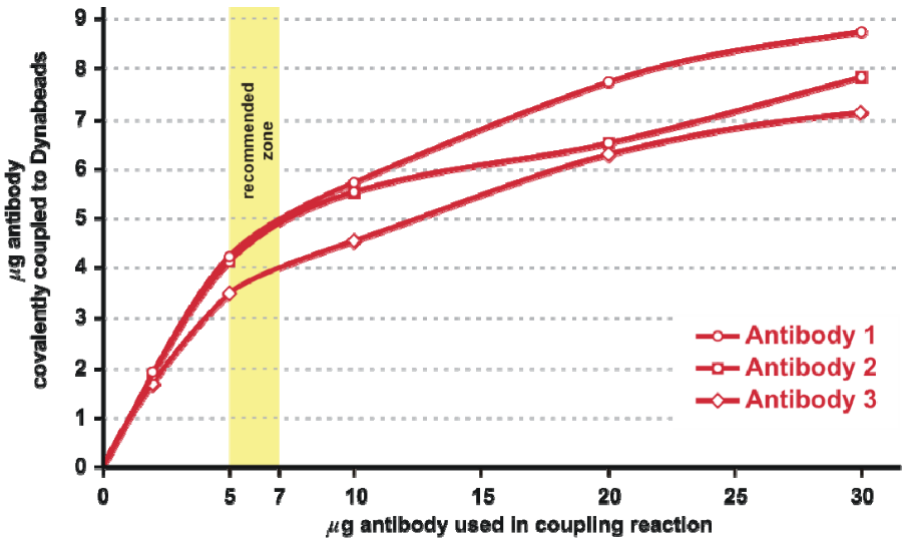
520 euro





Esempio di protocollo

5-7 µg di Ab per mg di biglia (> per Ab con scarsa avidità).



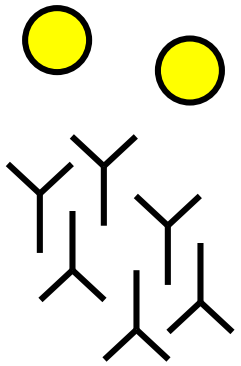
DA EVITARE O RIDURRE

- Sodium Azide (NaN_3)
- Antibody Stabilizing Proteins
  - Bovine Serum Albumin (BSA)
  - Gelatin
- Glycerol

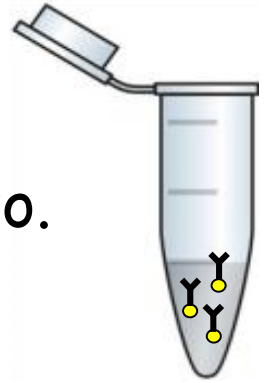
GIORNO 1

1. Lavaggio biglie e omogenizzazione.
2. 1' di magnete, eliminazione lavaggio.
3. Aggiunta Ab in opportuno tampone.
4. Incubazione a 37°C, 16-24h.

Dynabeads®

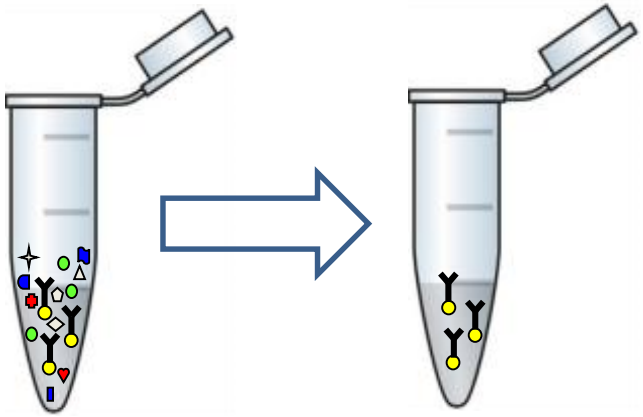


Protocollo semplificato



GIORNO 2

1. 1' di magnete ed eliminazione Ab non legato.
2. Lavaggio e omogenizzazione.
3. 1' di magnete, eliminazione lavaggio.
4. Aggiunta di un nuovo tampone per stabilizzare l'Ab.
5. Lavaggio e omogenizzazione.
6. 1' di magnete, eliminazione lavaggio.
7. Agitatore rotante per 15' a T ambientale.
8. 1' di magnete, eliminazione lavaggio.
9. Risospensione in 100µL di tampone e conservazione a 4°C.



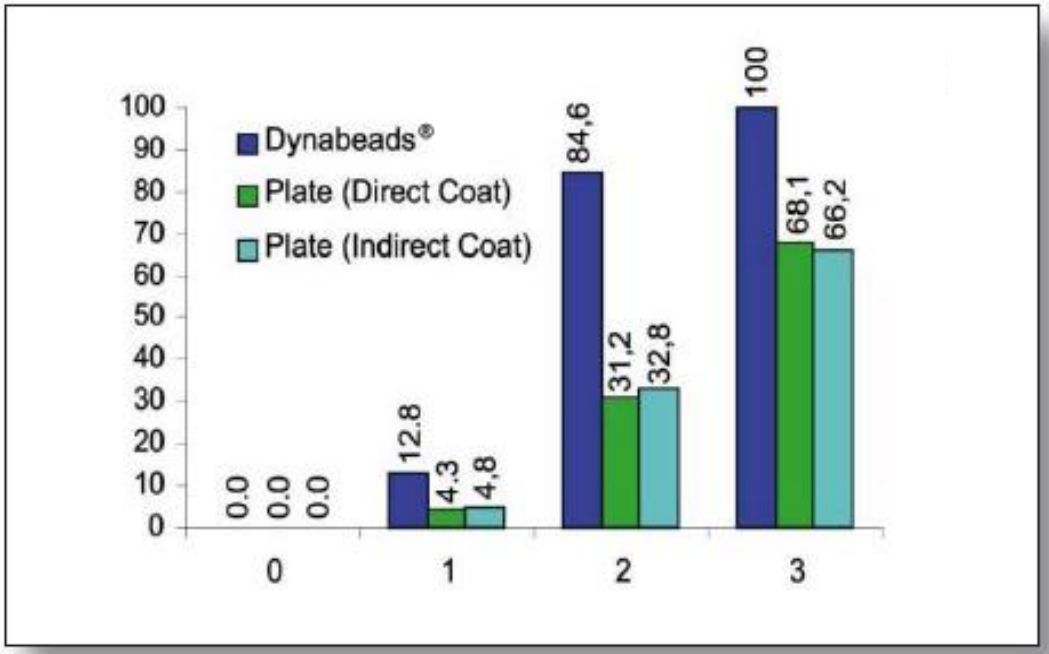
10. Aggiunta della miscela campione.
11. Incubazione OTTIMALE.
12. Lavaggio e omogenizzazione.
13. 1' di magnete, eliminazione lavaggio.

Separazione manuale od automatica

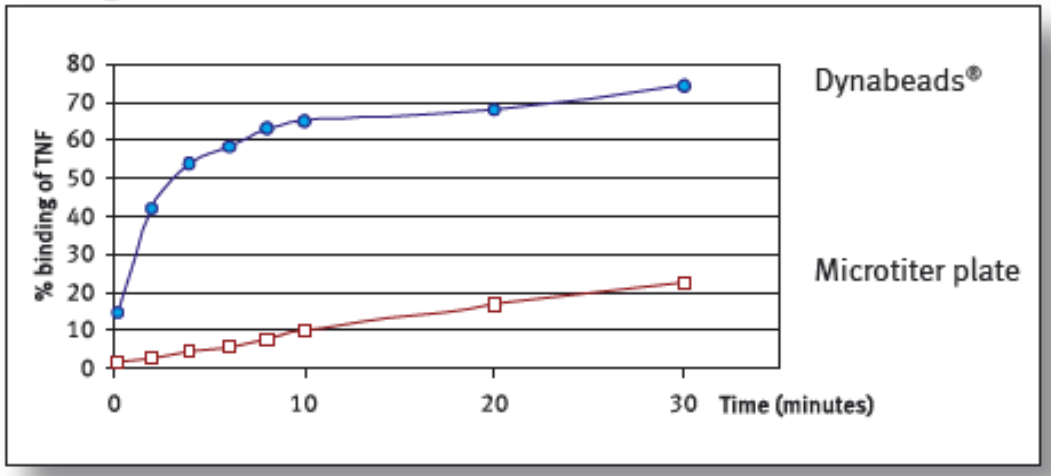
Automated protocols for high throughput screening are available for KingFisher® 96 from Thermo Electron.

Automatizzabile!

Confronto con metodi basati sull'uso di micropiastre.

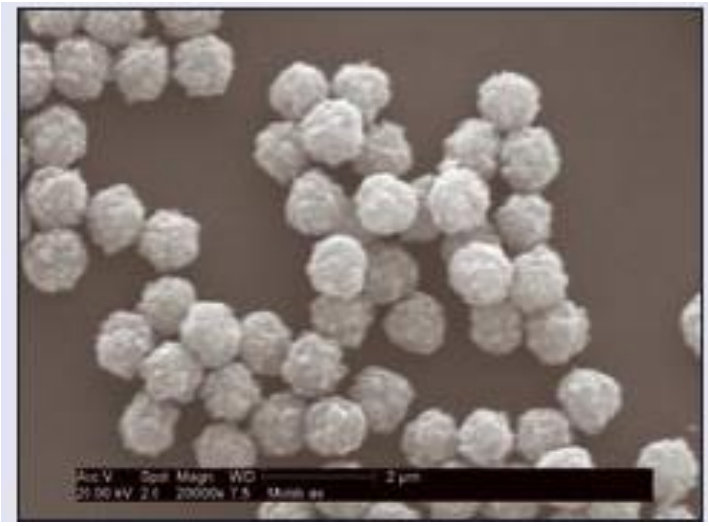


Confronto del legame di un Ab alla micropiastra o alle biglie.

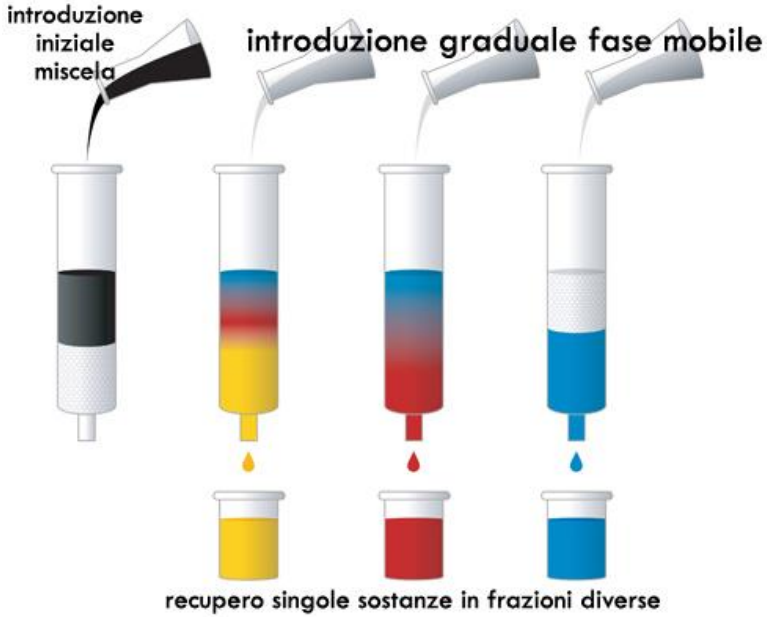


Cinetiche di legame del TNF.

Dynabeads vs Cromatografia

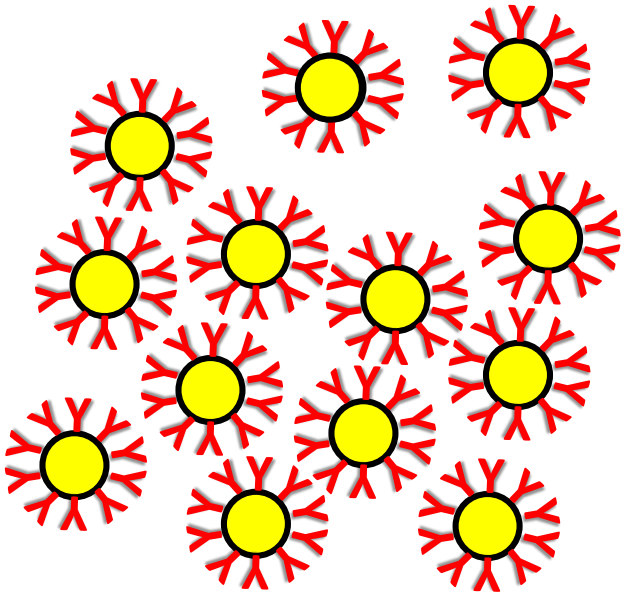


vs

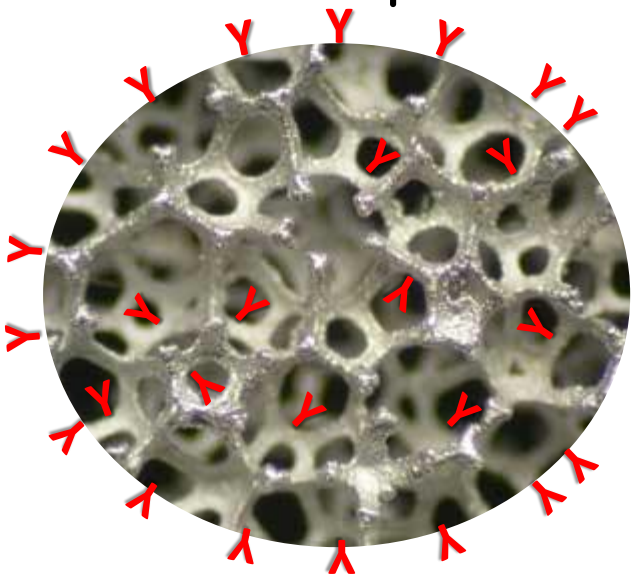


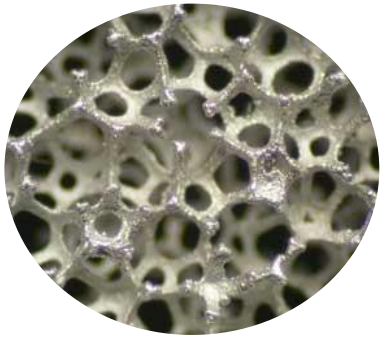
Dynabeads vs Cromatografia

Dynabeads®

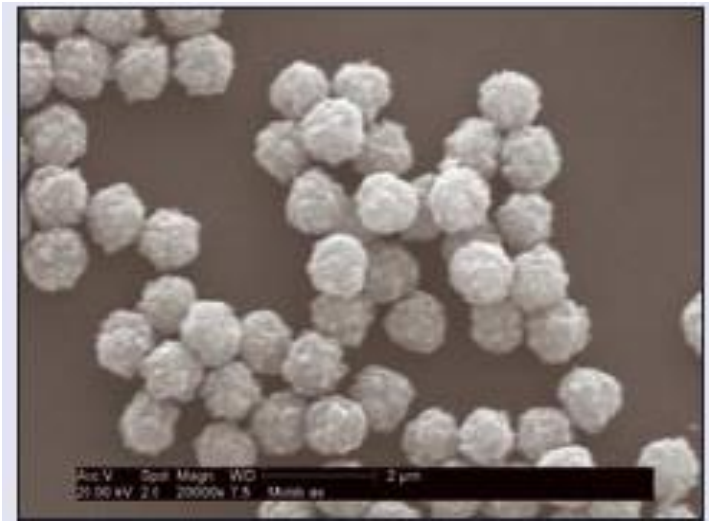


Materiale poroso





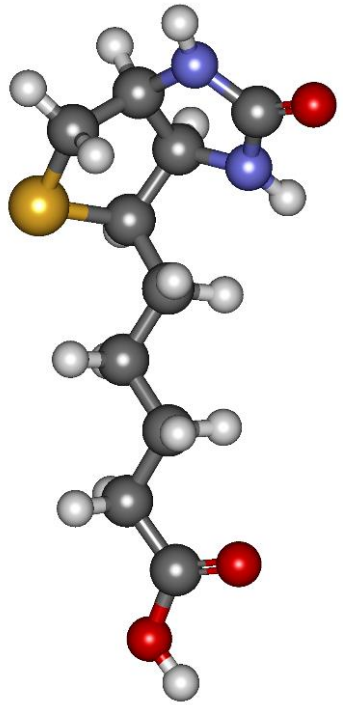
Dynabeads vs Cromatografia



vs

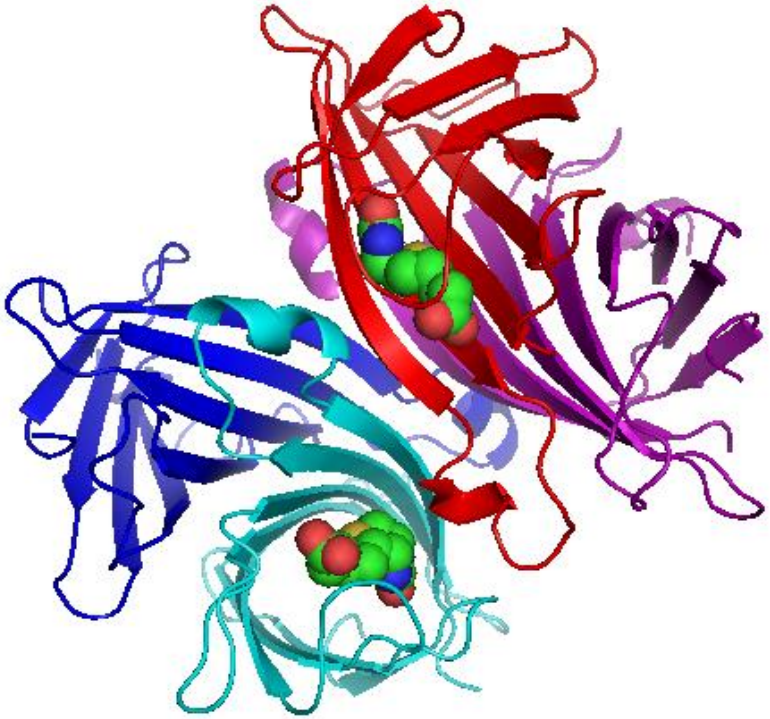
Streptavidina e Biotina

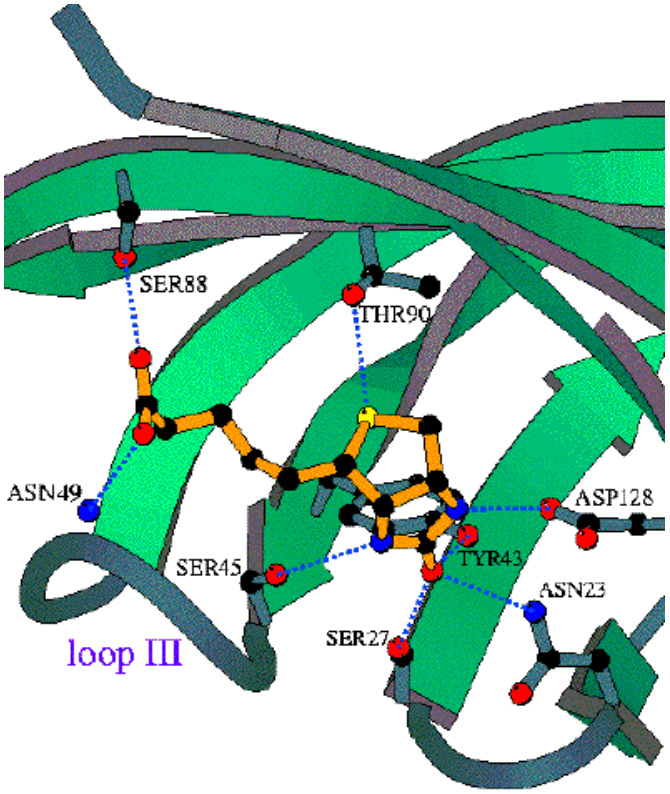
La STREPTAVIDINA è una proteina (MW 60 Kd) isolata dal batterio *Streptomyces avidinii*.



La BIOTINA è una vitamina idrosolubile.

Streptavidina e Biotina



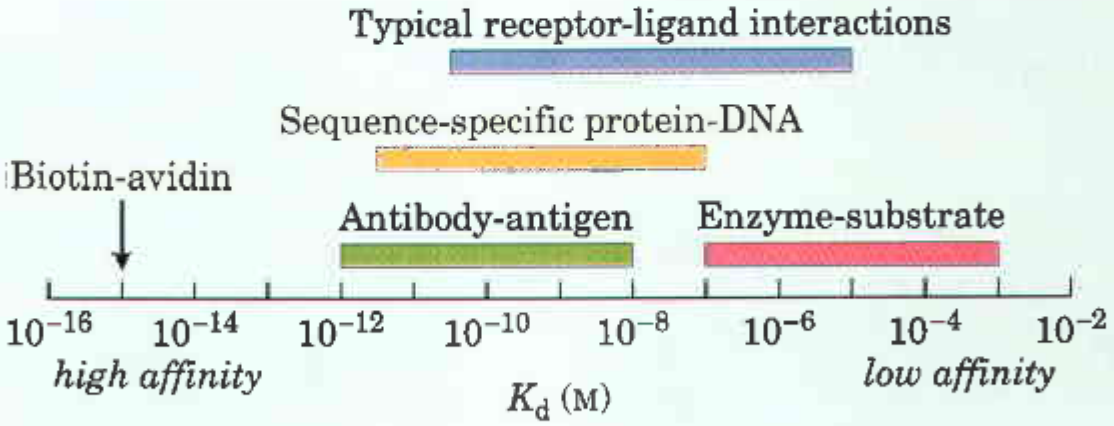


La streptavidina possiede 4 siti di legame ad alta affinità per la biotina.

Kd STREPTAVIDINA-BIOTINA

TABLE 5–1 **Some Protein Dissociation Constants**

Protein	Ligand	K_d (M)*
Avidin (egg white)	Biotin	1×10^{-15}
Insulin receptor (human)	Insulin	1×10^{-10}
Anti-HIV immunoglobulin (human)†	gp41 (HIV-1 surface protein)	4×10^{-10}
Nickel-binding protein (*E. coli*)	Ni^{2+}	1×10^{-7}
Calmodulin (rat)‡	Ca^{2+}	3×10^{-6}
		2×10^{-5}



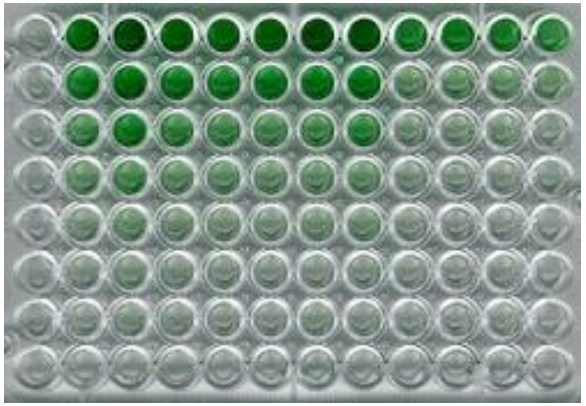
Color bars indicate the range of dissociation constants typical of various classes of interactions in biological systems. A few interactions, such as that between the protein avidin and the enzyme cofactor biotin, fall outside the normal ranges. The avidin-biotin interaction is so tight it may be considered irreversible. Sequence-specific protein-DNA interactions reflect proteins that bind to a particular sequence of nucleotides in DNA, as opposed to general binding to any DNA site.

Tyramide Signal **Amplification** Systems

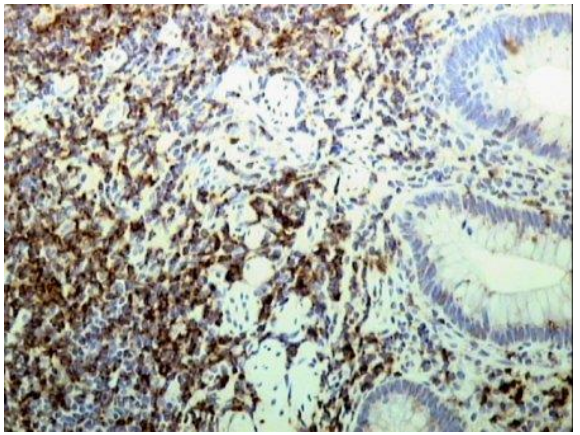
oppure

METODO **TSA**

ELISA

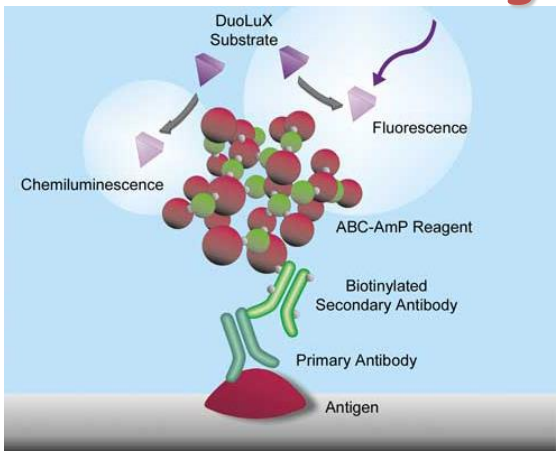


Immunoistochimica

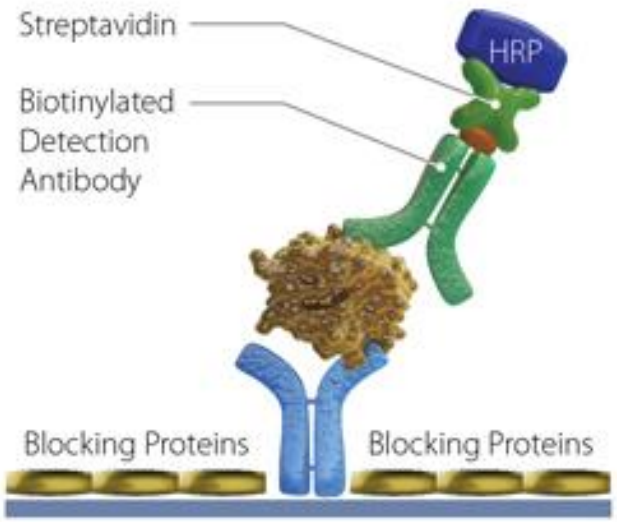


METODO TSA

Western Blotting



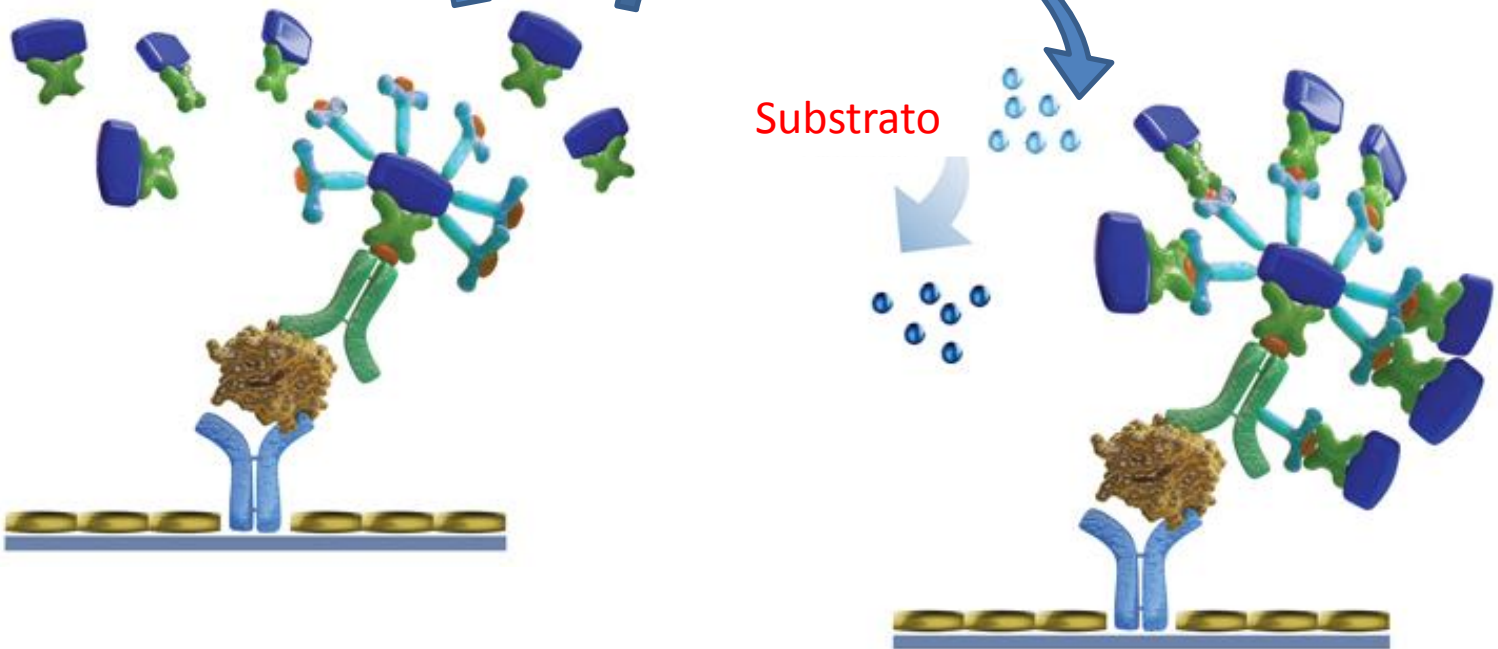
Amplificazione con Tyramide in ELISA



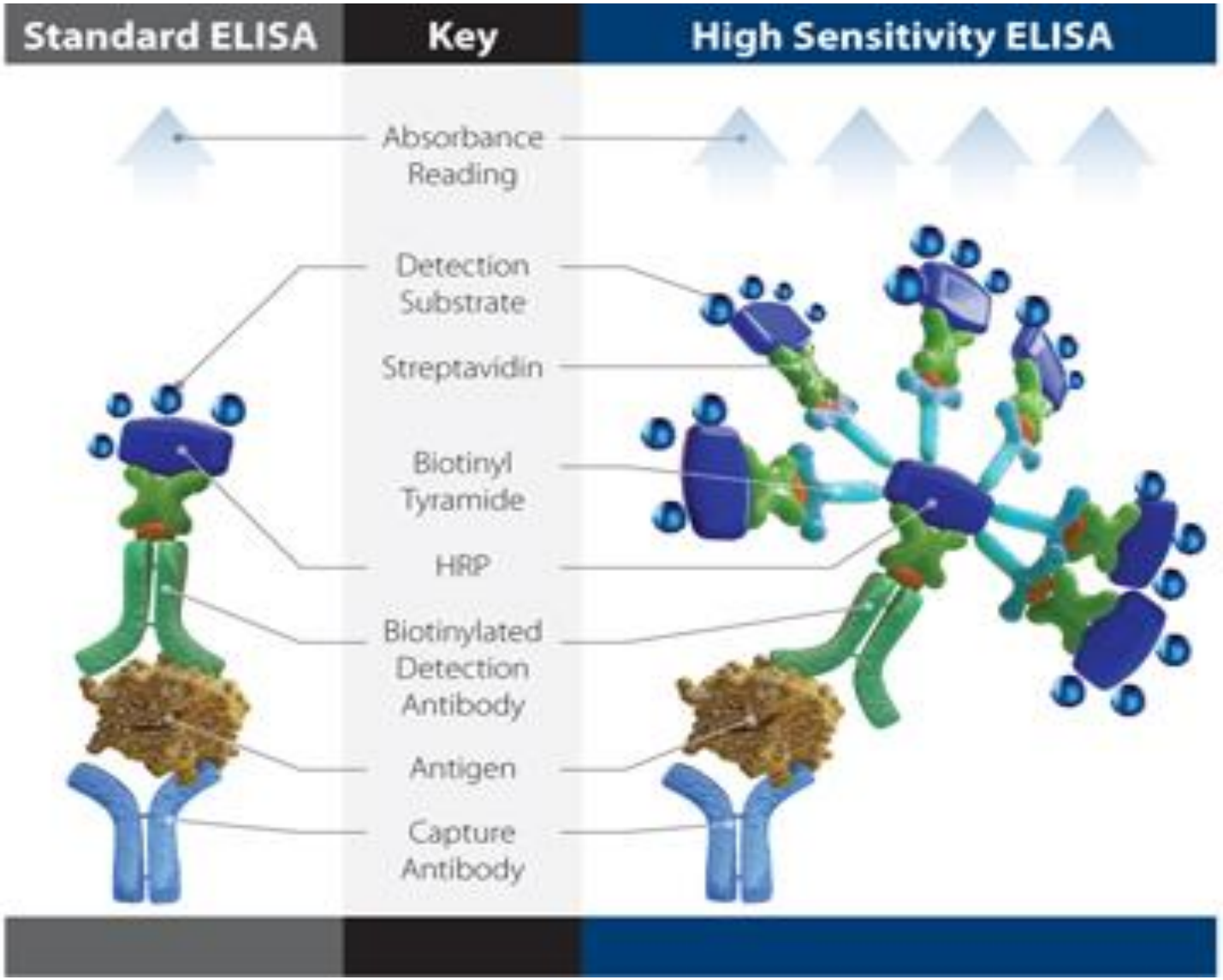
Il sistema TSA è usato per **aumentare** la sensibilità (**8-25 x**) e ridurre il consumo di Ab per la rilevazione.

Tyramide biotinilata

Substrato

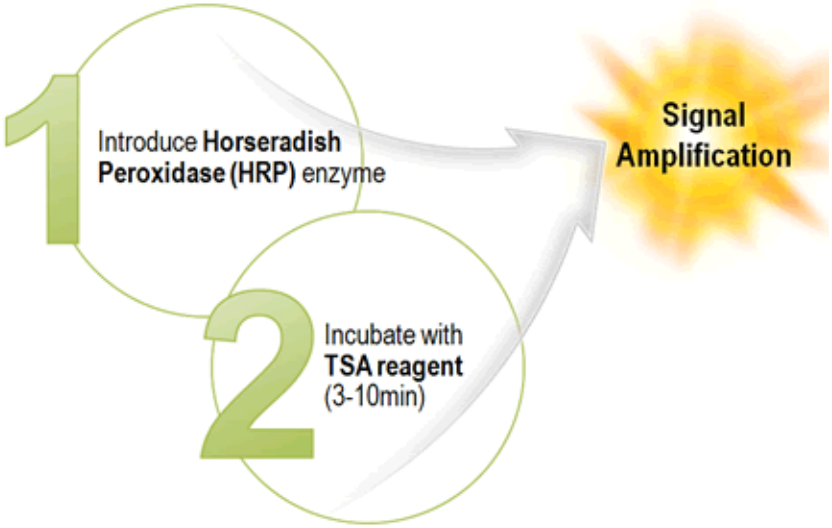


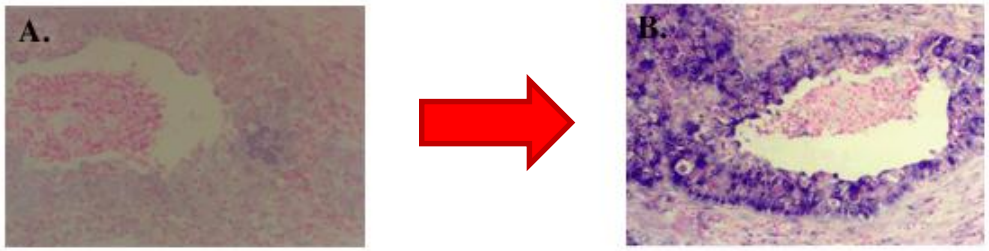
Amplificazione con Tyramide in ELISA

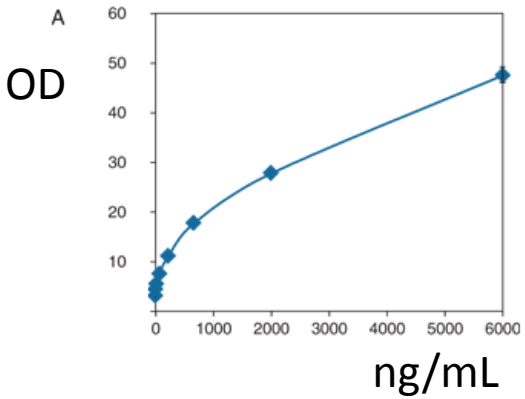


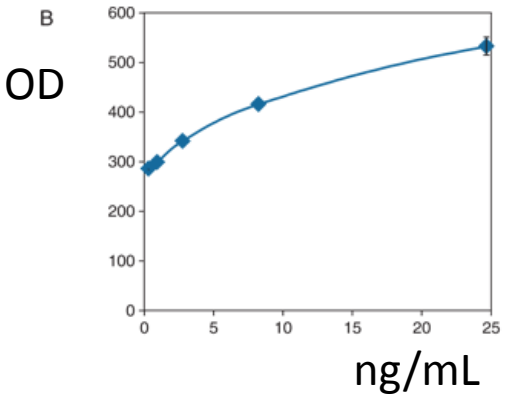
http://las.perkinelmer.com/Catalog/ProductInfoPage.htm?ProductID=NEP116E001EA

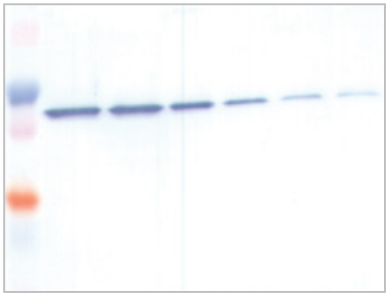
Amplificazione con Tyramide in ELISA



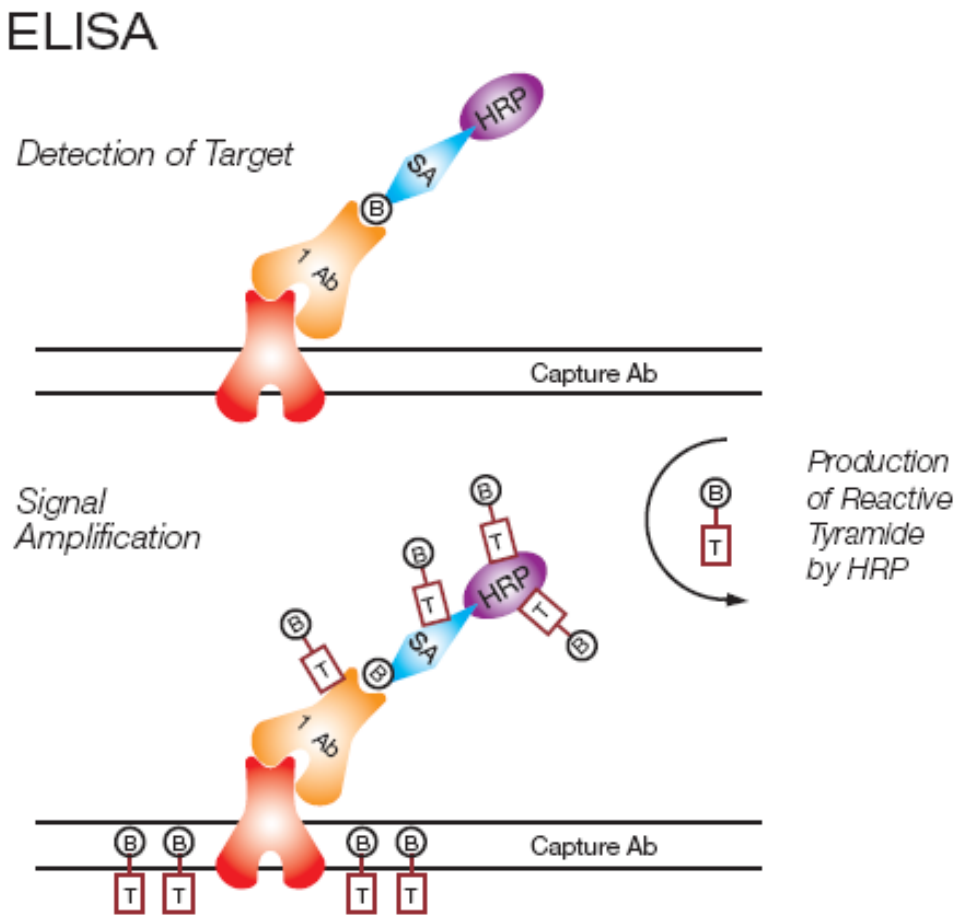






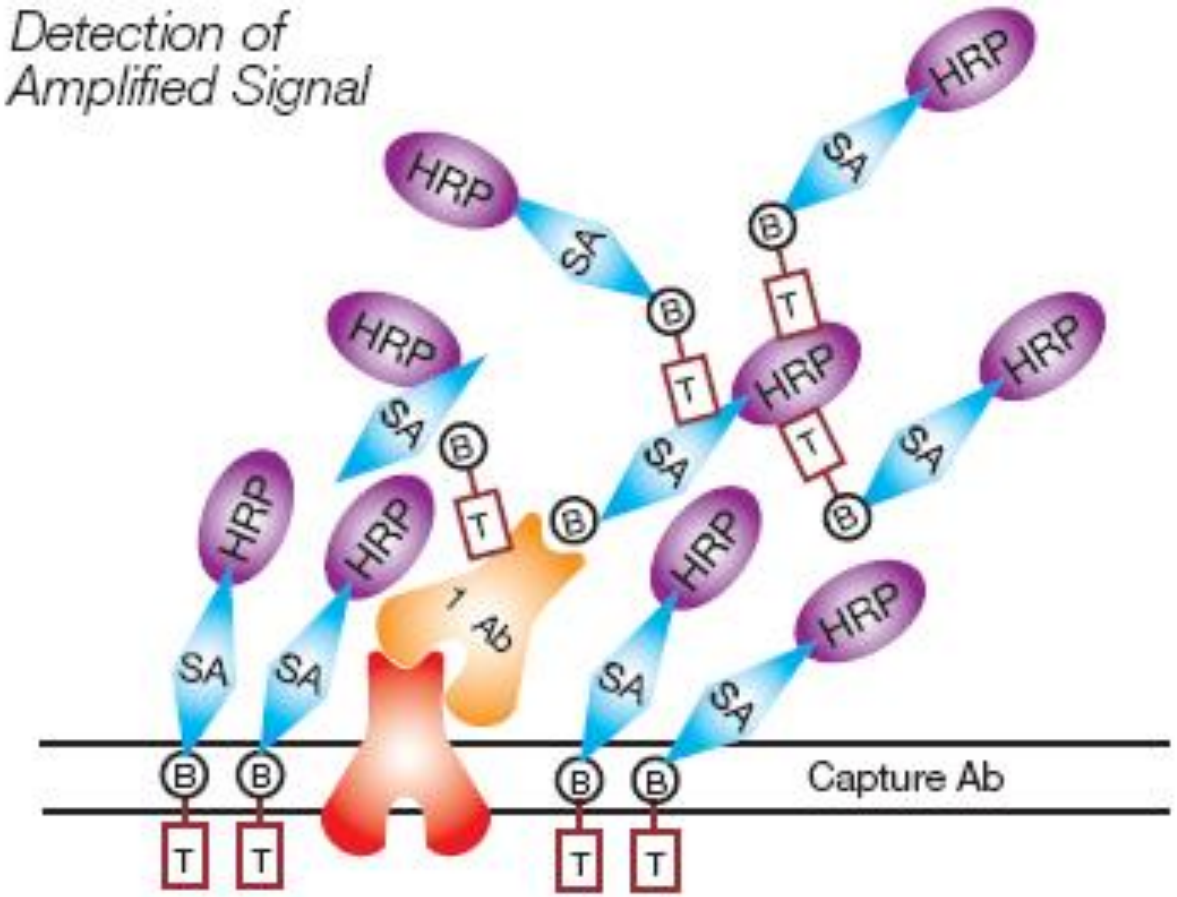


Fasi aggiuntive



Amplification and Visualization Procedures	Actual Time Added to Protocol
Blocking Step	30 min
Wash Step	4 x 5 min
Incubation of biotinyl-tyramide	3–10 min
Wash Step	4 x 5 min
Addition of enzyme conjugate	30 min
Wash Step	4 x 5 min
Incubation of chromogen	

Principio e limite



- HRP e H_2O_2 convertono la tiramide in una molecola reattiva

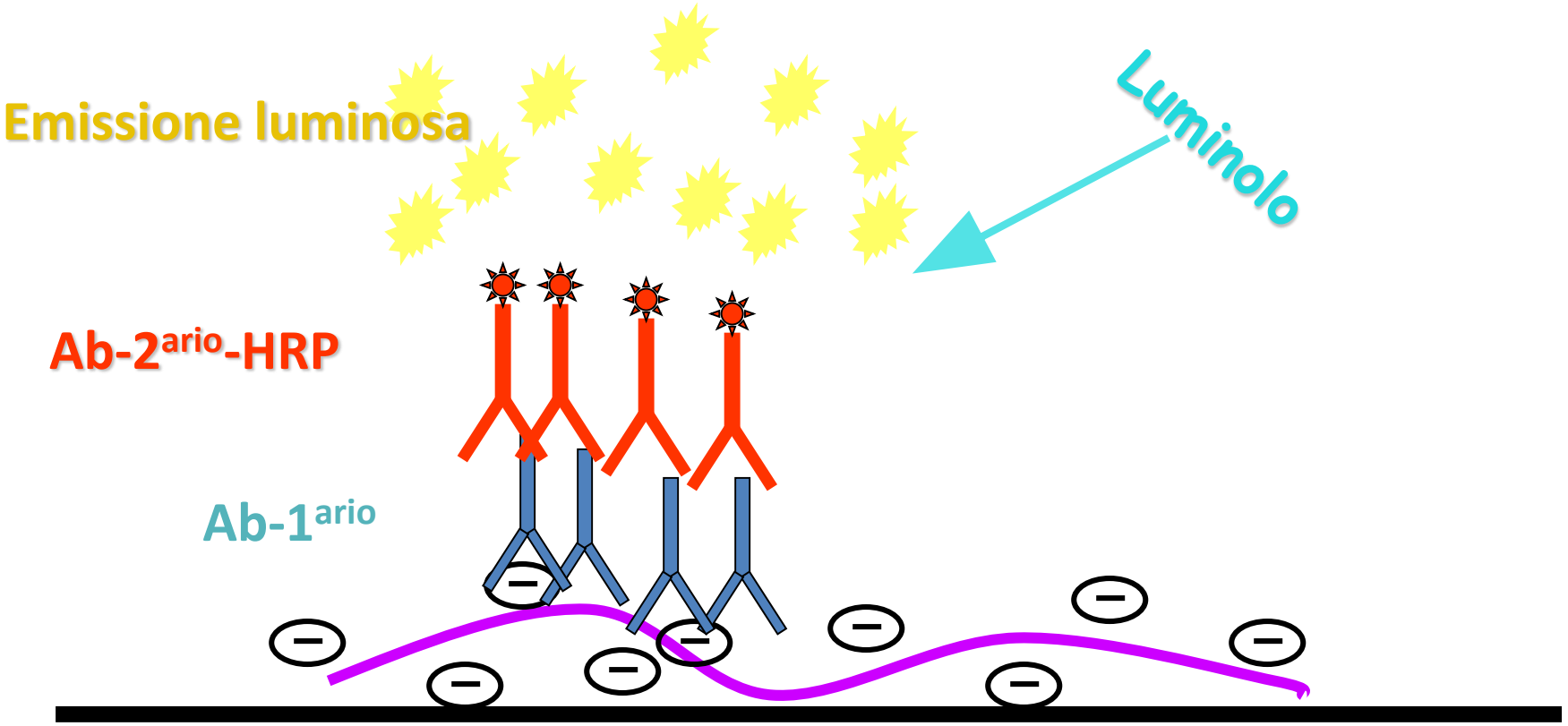
- Legami covalenti

Problema del background

Western Blot

Enzima marcatore = Perossidasi (HRP)

Substrato = Luminolo



In presenza di perossidasi e H_2O_2 il luminolo viene **ossidato**: si produce luce, la cui intensità può esser aumentata di 1000 volte con un intensificatore chimico.

Western BLAST®, 500 cm2

Product number: NEL761A001KT

PerkinElmer

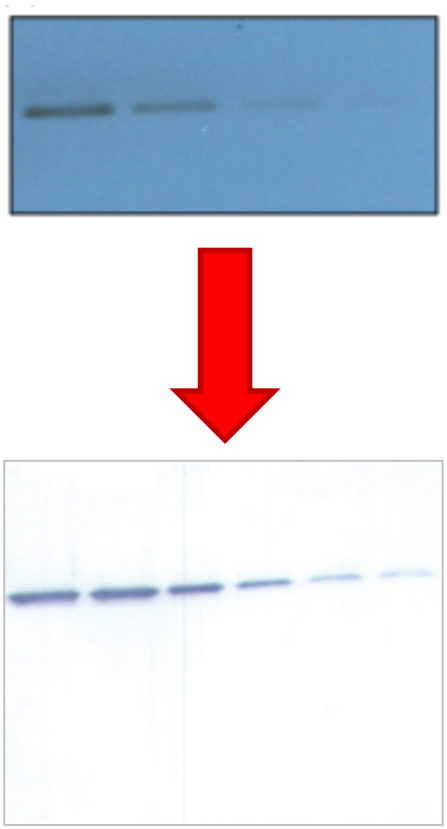
Sempre basato sul sistema TSA

-Trasforma il WB in tecnica **colorimetrica**.

- **Non** necessita di camera oscura.

- > riproducibilità.

- Sensibilità paragonabile alla luminescenza (amplificazione del segnale di 8-10 volte).



Pregi

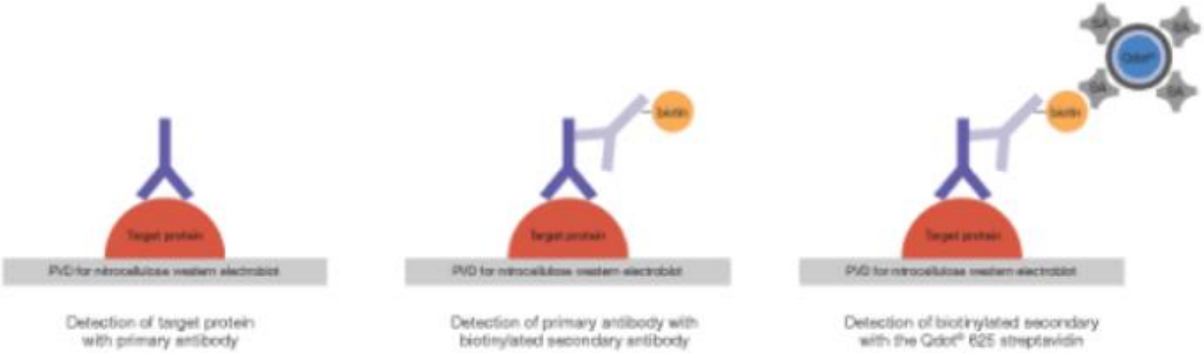
Permette:

- Di usare quantità ridotte di anticorpi.
- La riduzione dei tempi di incubazione.
- L'amplificazione di segnali deboli.
- Di rendere colorimetriche reazioni luminescenti

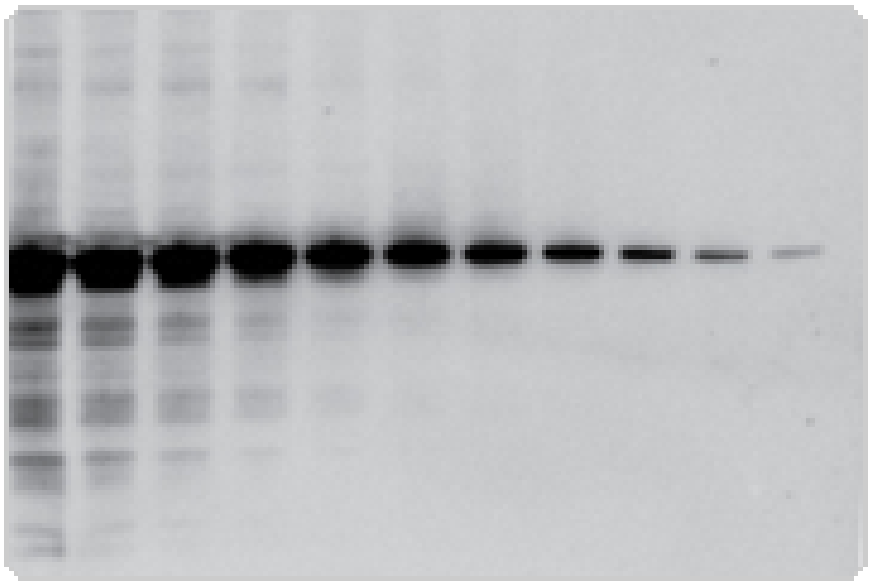
Difetti

Il rumore di fondo

Concorrenti….



WesternBreeze®
Chromogenic Western Blot Immunodetection Kit
Catalog nos. WB7103, WB7105, WB 7107



invitrogen™