

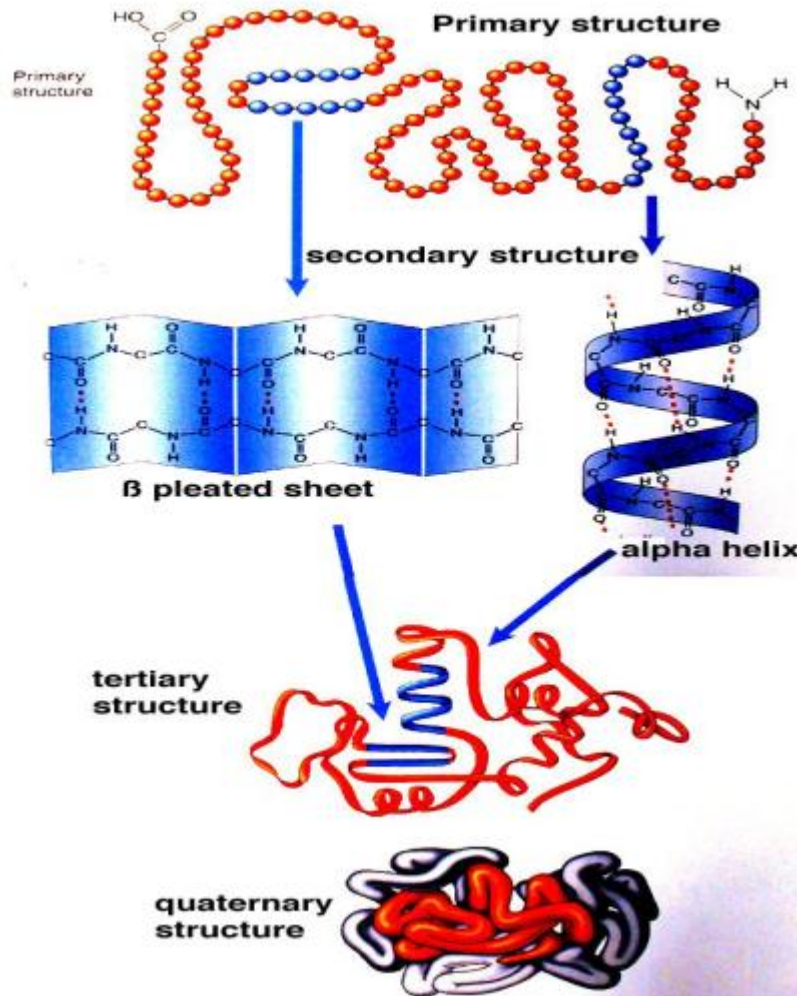


# **PROTEİN ANALİZ YÖNTEMLERİ; WESTERN BLOT ve ELİSA**

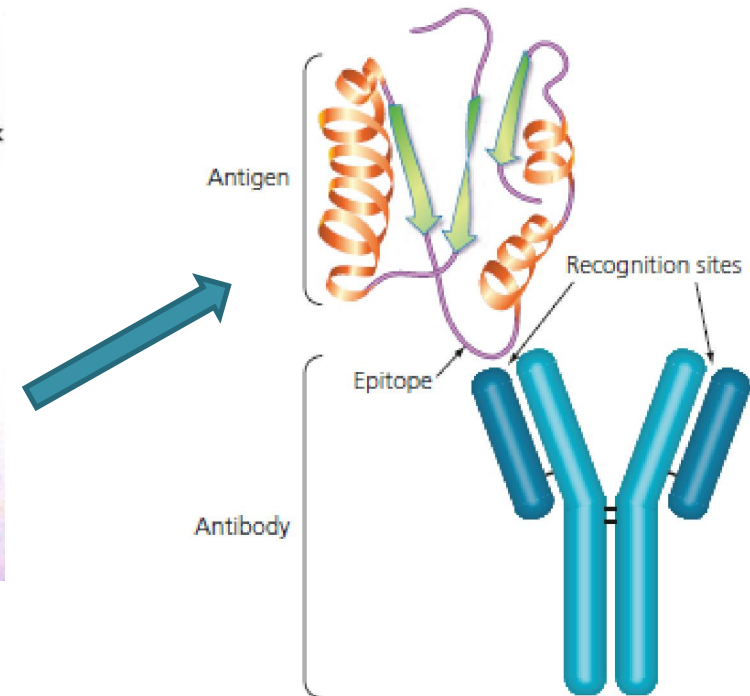
# PROTEİN ANALİZ YÖNTEMLERİ

- ELISA “Enzyme-linked immunosorbent assay”
- Western Blot
- Elektroforez
- Kromatografi
- Protein parmakizi
- X-Işını Kristalografisi
- Nükleer Manyetik Rezonans (NMR)
- Kütle spektrometresi (Mass spectrometry, MS)
- Protein mikroarray

# Protein-Antikor İlişkisi



- Proteinler 3D
- 3D; kendine has antijenik determinant



# ELISA

- **ELISA** (**E**nzyme-**L**inked **I**mmuno**S**orbent **A**ssay)
- Biyokimyasal-İmmünolojik bir yöntemdir
  - Örneklerdeki antikor ve antijen tayininde
- Çok duyarlıdır (pg/mL)

# Nerelerde kullanılır

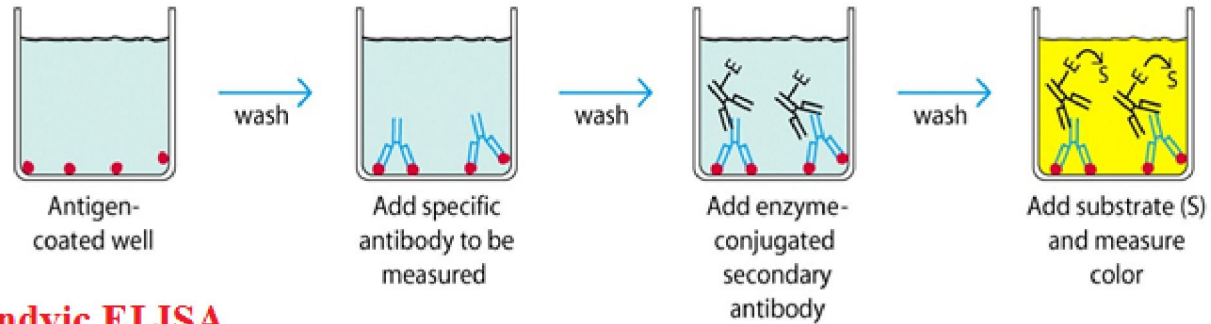
- Araştırma amaçlı
- Serum Antikor Konsantrasyonu
- Potansiyel gıda alerjilerinde
  - (süt, yer fıstığı, ceviz, badem, yumurta vb.)
- Salgın hastalıklar ve yayılımının belirlenmesi
  - HIV, kuş gribi, grip, kolera vb.
- Antijen tayini
  - Hamilelik hormonu, ilaç allerjisi, deli dana hastalığı vb.
- Geçirilmiş hastalıkların antijenlerinin kandan tayini
  - HIV, kuş gribi vb.

# Nelerden ELISA Çalışabiliriz?

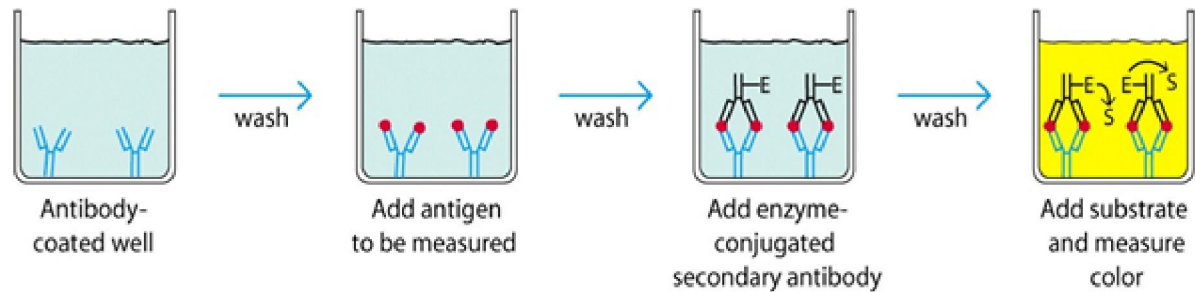
- Vücut sıvılarından
  - Serum
  - Plazma
  - BAL
  - Plevra sıvısı
  - Nefes havası vb.
- Hücre Kültürü
  - Süpernatant
  - Hücre lizati
- Doku (homojenizasyon..!)

# ELISA Çeşitleri

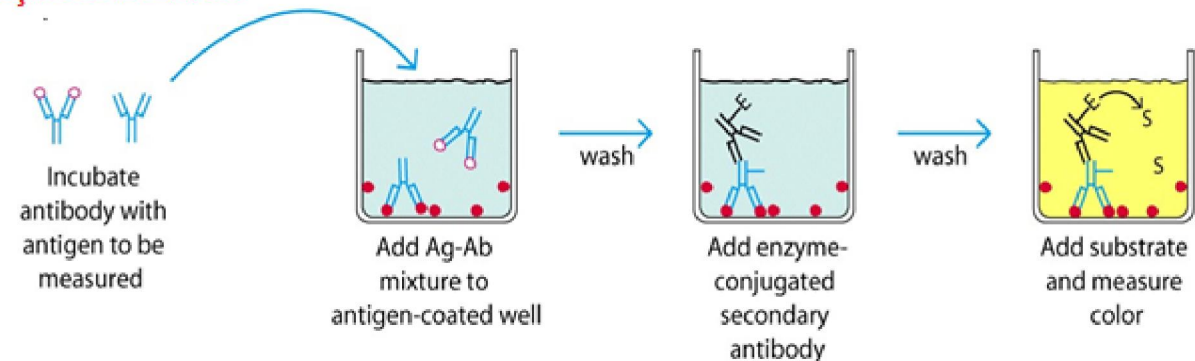
## İndirek ELISA



## Sandviç ELISA



## Yarışmalı ELISA

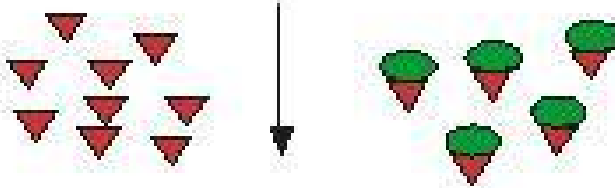


# Yarışmalı ELISA

## Competitive Enzyme Immunoassay



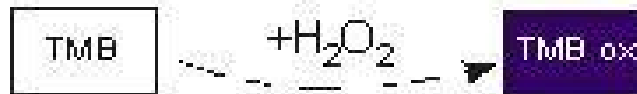
Solid phase coated with antibody



Add free and labelled antigen



Free and labelled antigen are captured

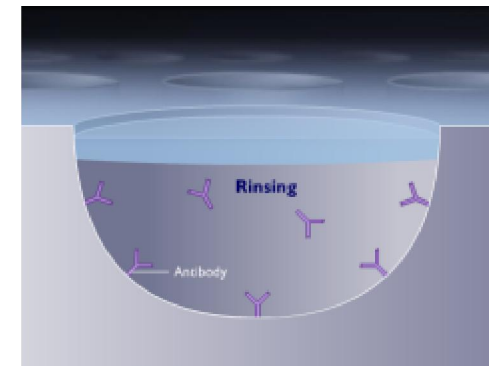
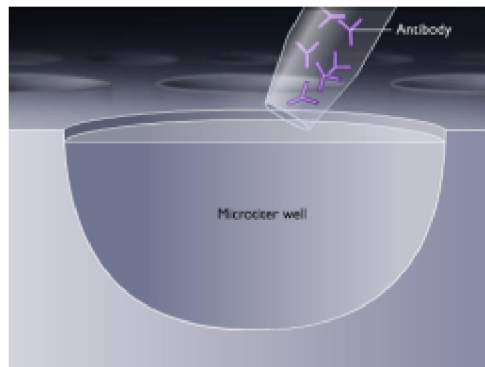
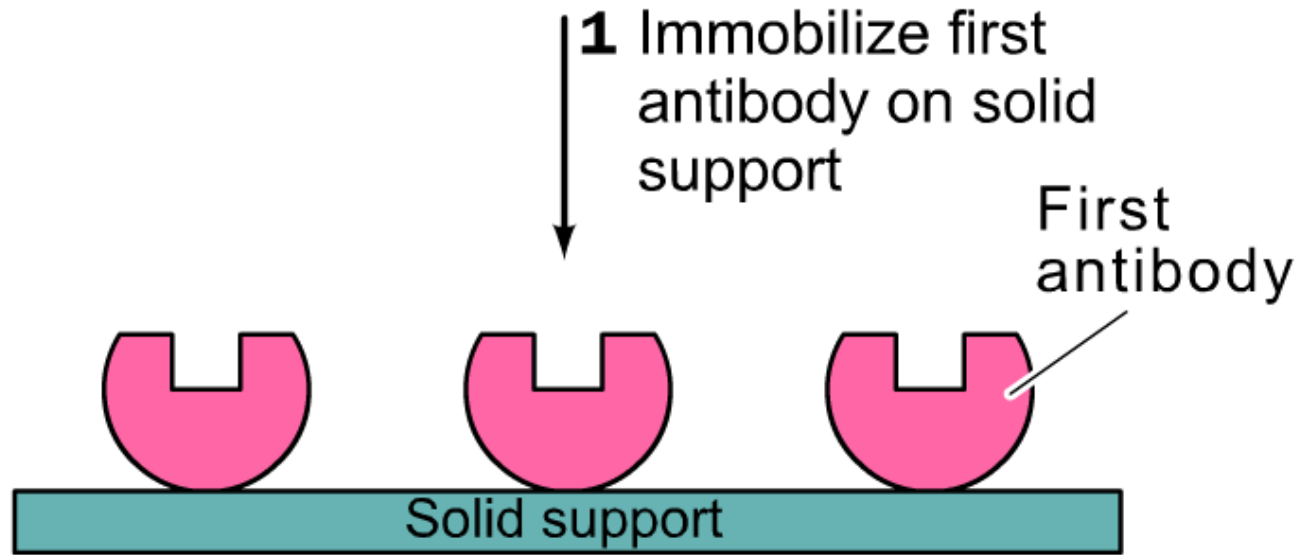


Color formation by oxidation of substrate into a colored compound



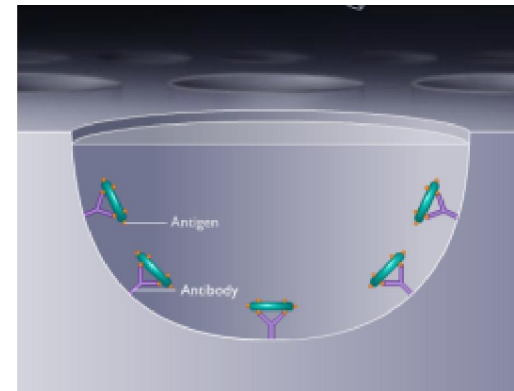
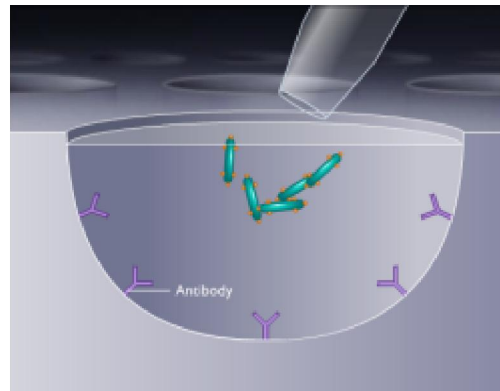
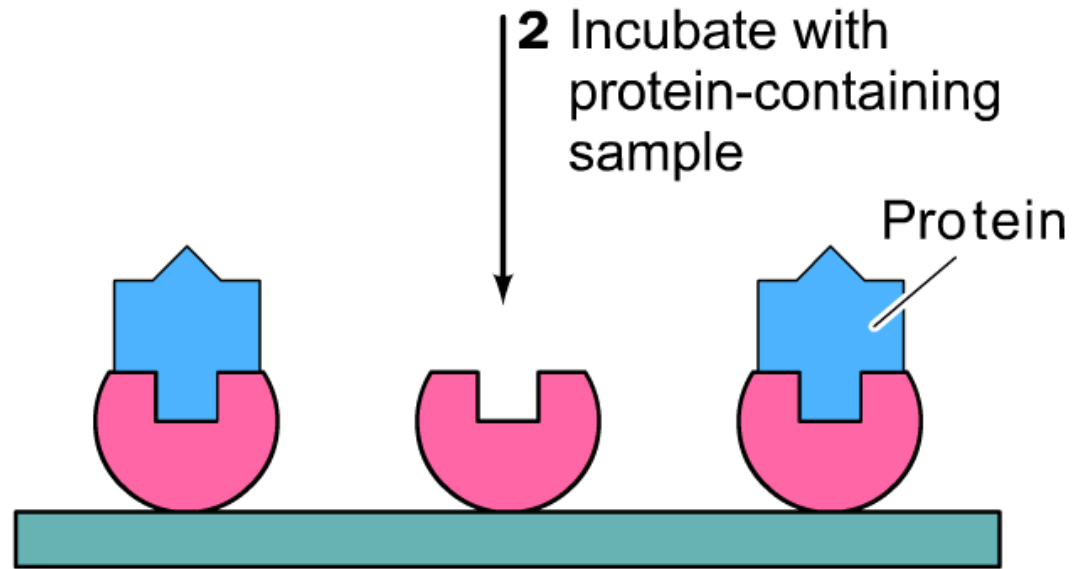
# Sandviç ELISA

- ELISA plate proteine özgü antikorla kaplanır



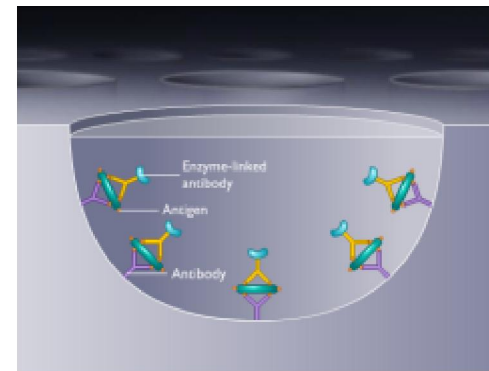
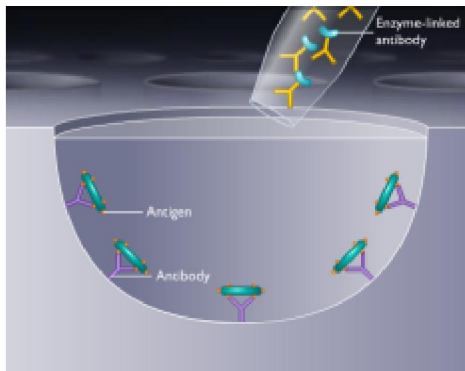
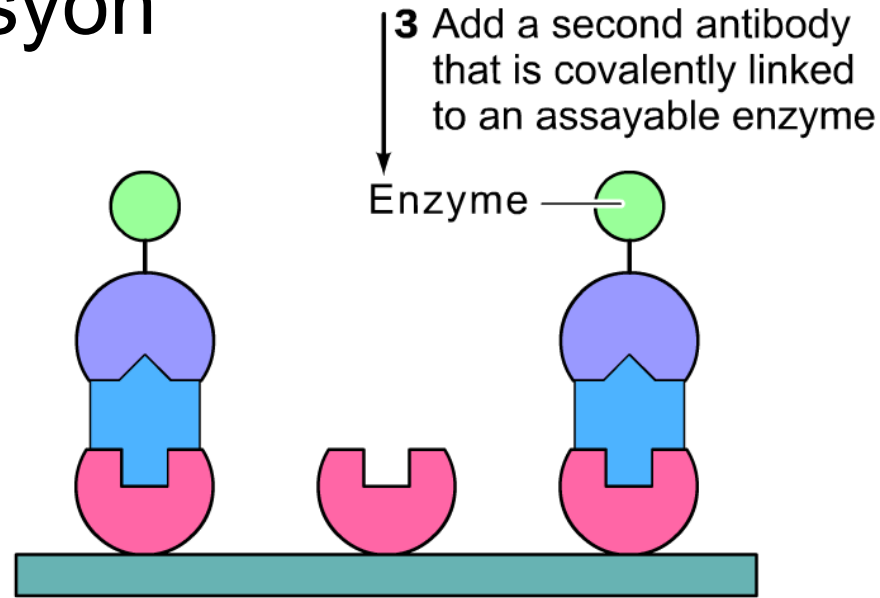
# Sandviç ELISA

- Protein içeren örneklerle inkübasyon



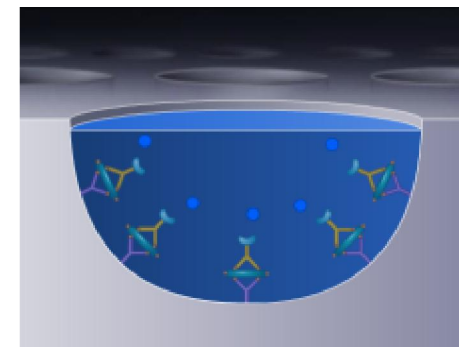
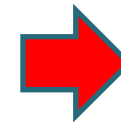
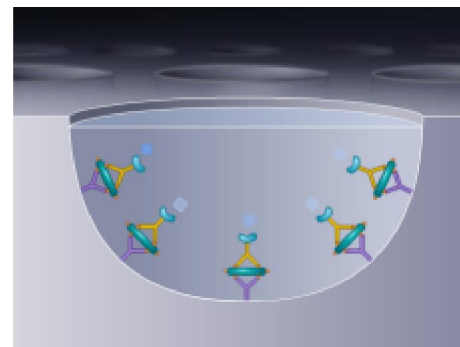
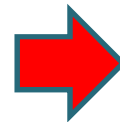
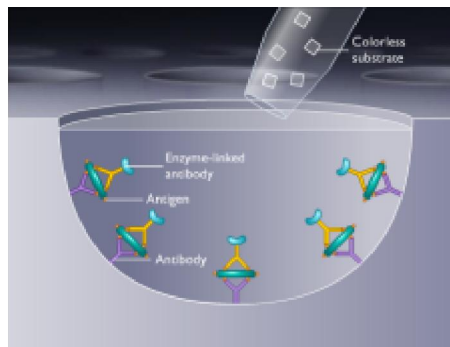
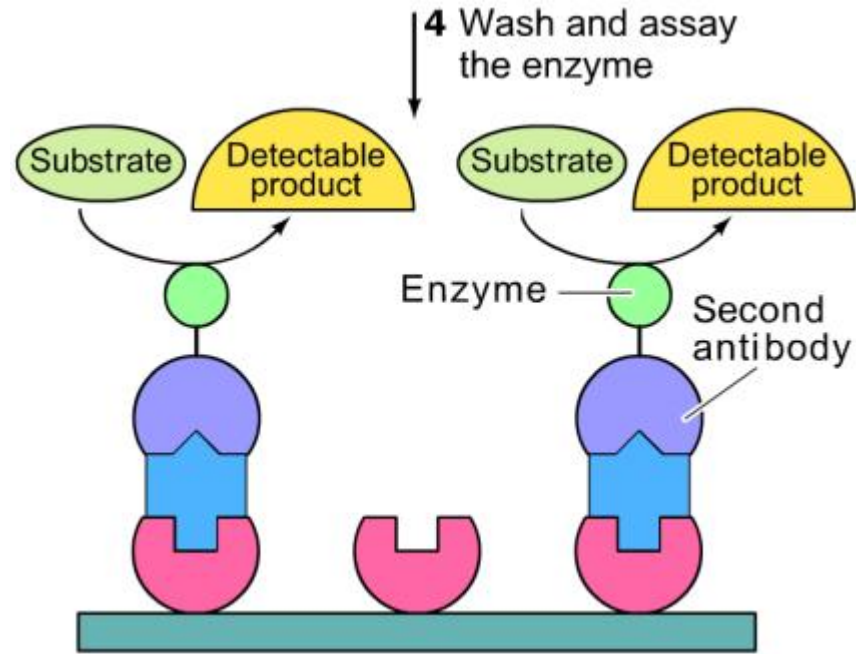
# Sandviç ELISA

- Enzim ekli sekonder antikor ile inkübasyon

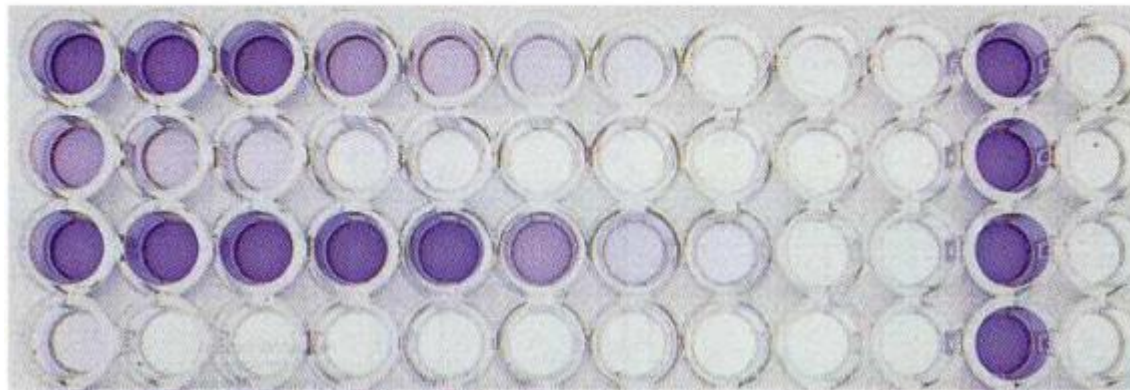
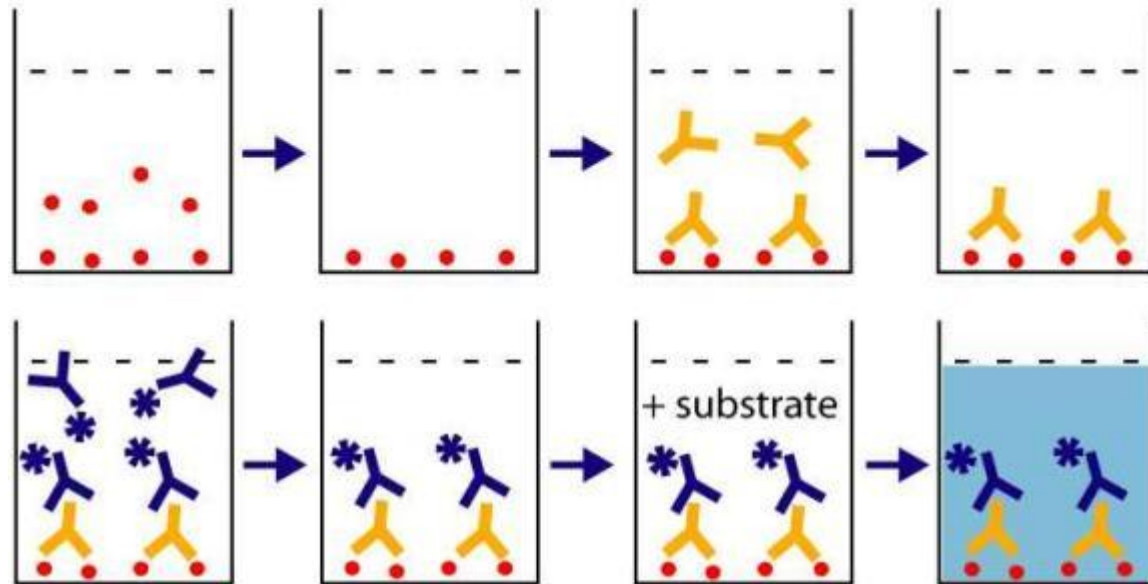


# Sandviç ELISA

- Enzim substratının eklenmesi ve renk değişimi



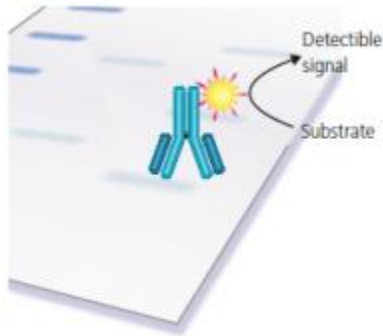
# İndirek ELISA



# Nelere Dikkat Edelim?

- Çalışma planı
  - Çalışılacak örnek
    - Serum
    - Plazma
    - Hücre kültürü
    - Nefes havası vb.
  - ELISA kiti seçimi
    - Hassasiyet
- Seyreltme/Yoğunlaştırma
- Uygun araç seçimi
  - Pipet
  - Pipet ucu

# Western Blot



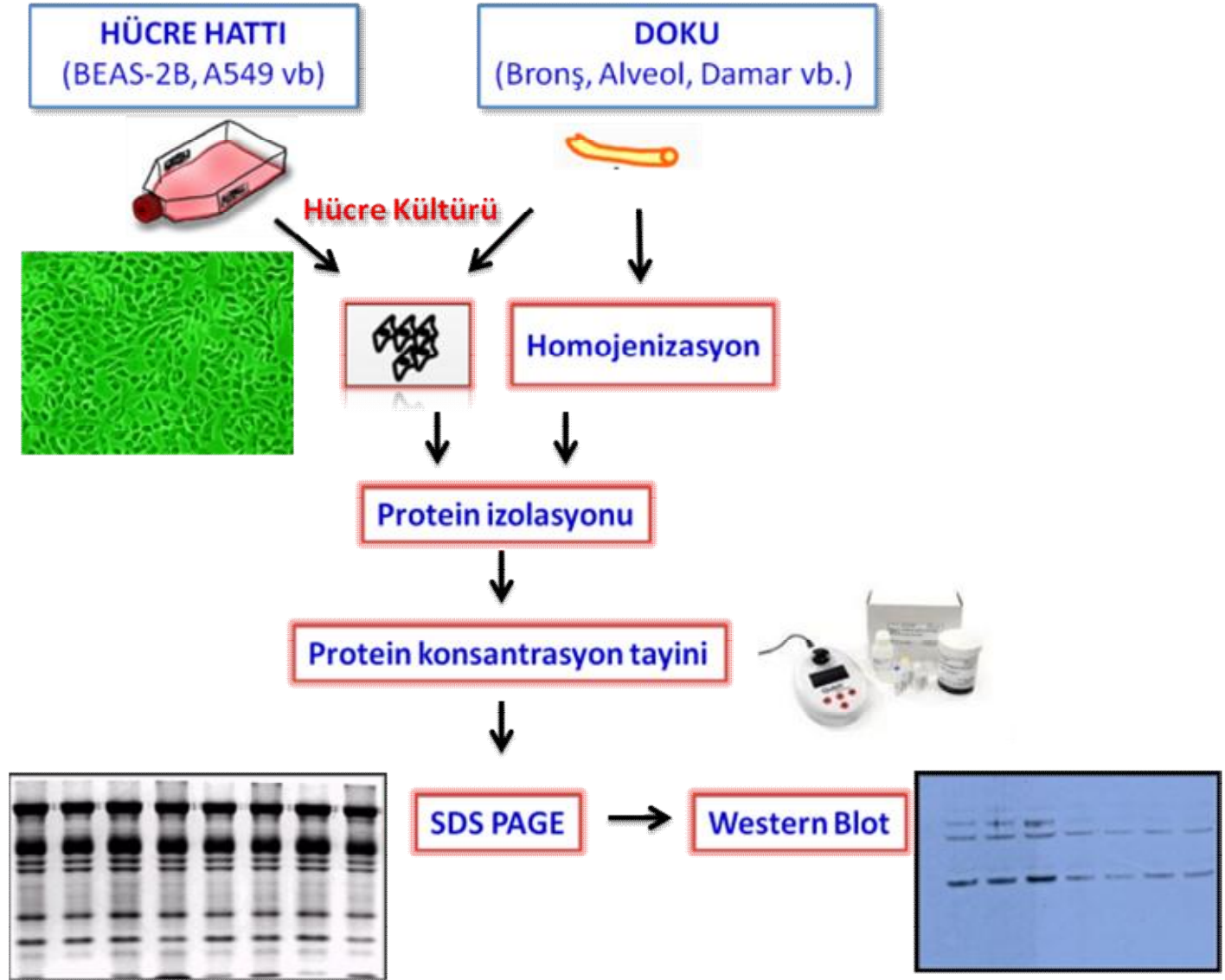
- **W**estern **B**lot (**WB**) testi moleküler biyolojide protein solüsyonunda aranan bir proteinin olup olmadığını ve varsa ne kadar olduğunu anlamak için kullanılan bir yöntemdir
- Southern Blot; (1975) (DNA/DNA-prop)
- Western Blot; Towbin (1979)

# WB Nereelerde Kullanılır?

- Kanser biyolojisi ve patolojisi
- Mikrobiyoloji
- İmmünoloji
- Protein biyokimyası
- Doku çalışmaları
- Antikor testlerinde



# WB



# WB

## Hücre Lizatı

Hücrelerden işaretlenmek istenen protein için uygun liziz çözeltisi ile protein lizatı elde edilir

## Jel Elektroforezi

SDS-PAGE ile proteinler ayrılır

## Proteinin jelden membrana transferi

Proteinler membran üzerine transfer edilir

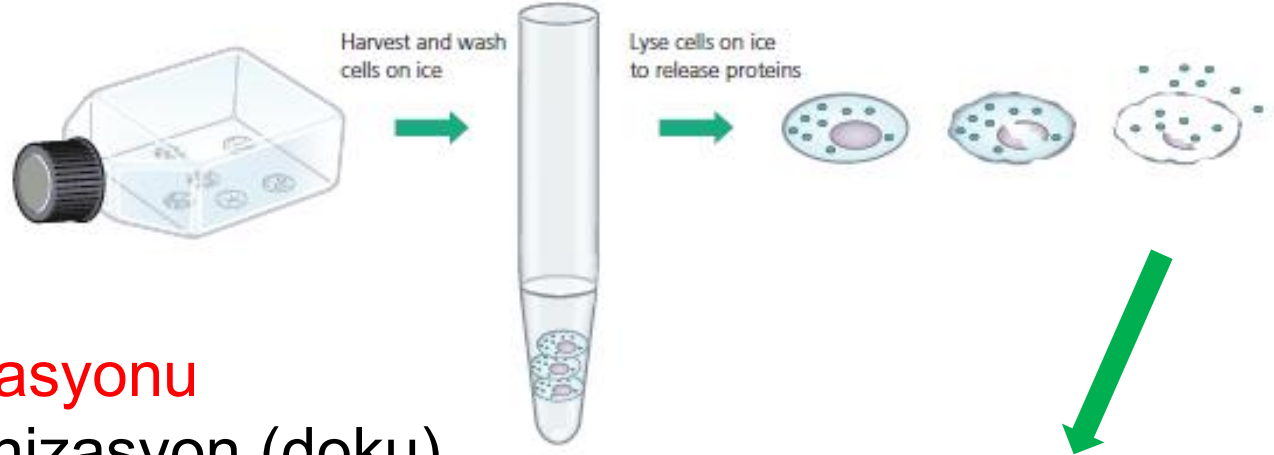
## İmmün blotlama (Antikor inkübasyonu)

Bloklama, primer antikor, sekonder antikor ilaveleri ve yıkama ile immünblotlama yapılır

## Proteinin belirlenmesi ve analizi

İlgili protein görünür ve miktar ölçülebilir hale getirilir

# Protein İzolasyonu ve SDS-PAGE

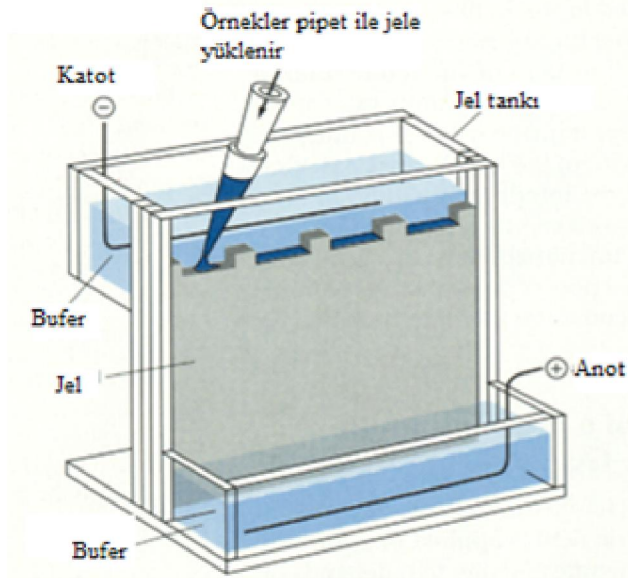


## •Protein İzolasyonu

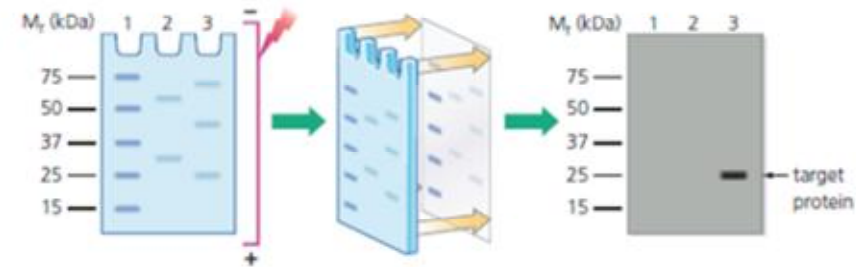
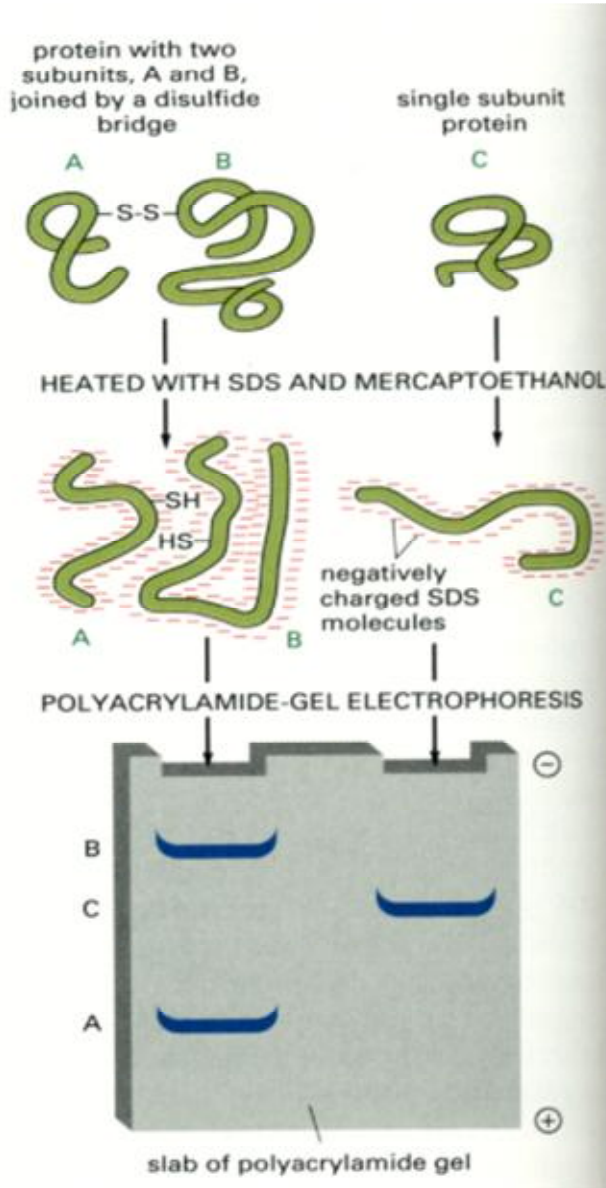
- Homojenizasyon (doku)
- Proteaz İnhibitörleri
- Liziz bufer

## •SDS-PAGE

- Her bir kuyucuğa aynı miktar protein
- Jel konsantrasyonu (%Akrilamit-bisakrilamit)
- Elektroforez süresi ve akımı

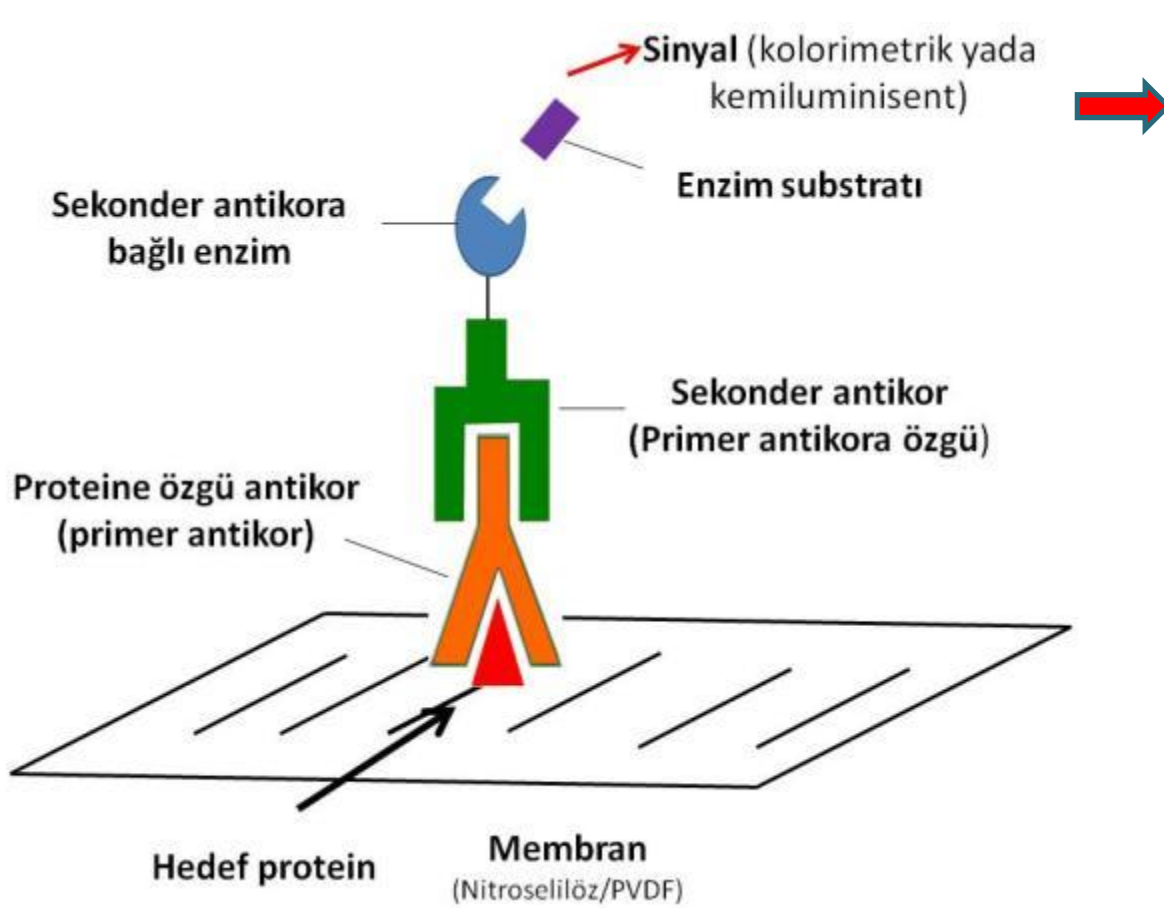


# SDS-PAGE ve Blotlama





# GÖRÜNTÜLEME



# Nelere Dikkat Edelim?

- Protein
  - İzolasyonu (Hücre kültürü/Doku)
  - Miktarının belirlenmesi
- SDS-PAGE
  - jel yoğunluğu
  - Elektroforez süresi ve akımı
- Blotlama
- Antikor seçimi
  - Aynı proteine özgü birden fazla antikor olabilir
- Optimizasyon
  - Primer antikot ile inkübasyon süresi ve sıcaklığı

# ELISA - Western Blot

- Her iki yöntemde de
  - Doku yada hücresel örneklerdeki spesifik proteinler belirlenir.
  - Antikor kullanılır (primer ve sekonder)
  - Yeni proteinlerin ve fonksiyonlarının belirlenmesinde kullanılması zorunludur

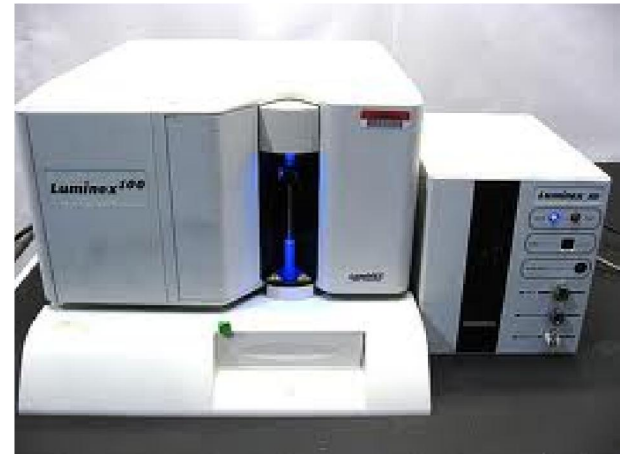


# Alternatif Yöntemler

- **Flow sitometri**



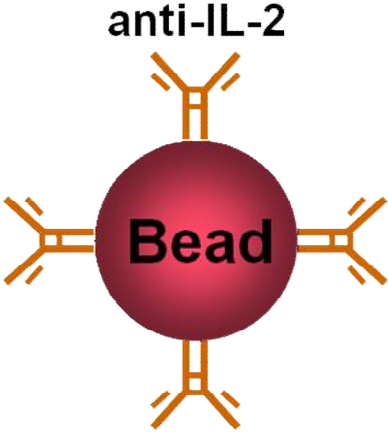
- **Luminex**



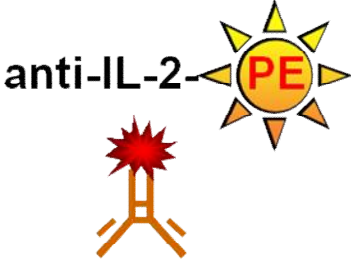
# Flow sitometri



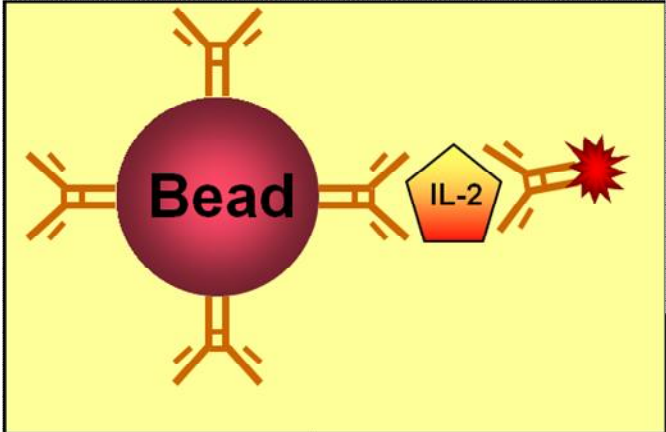
Soluble Analyte



capture bead

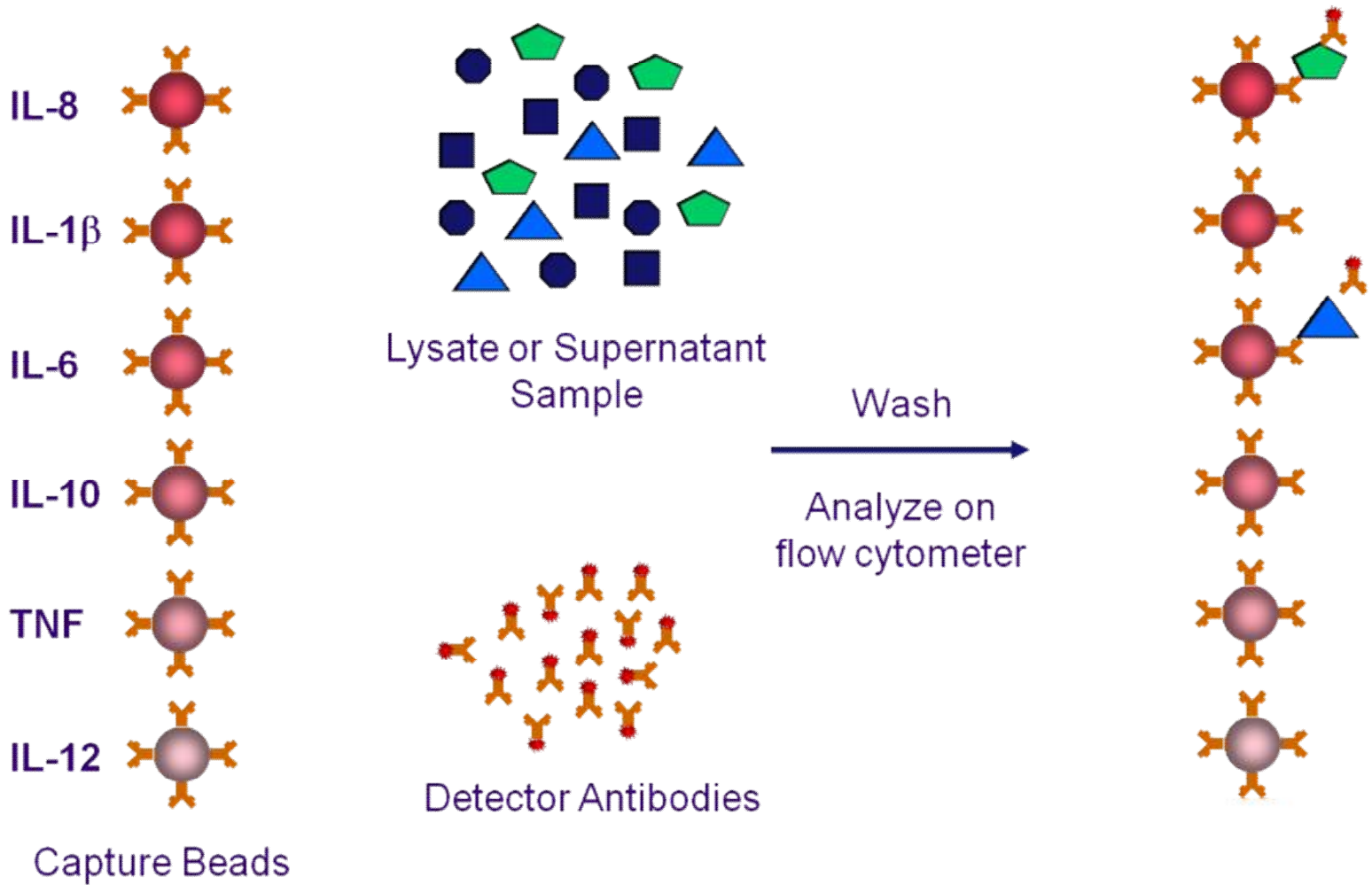


Detection antibody

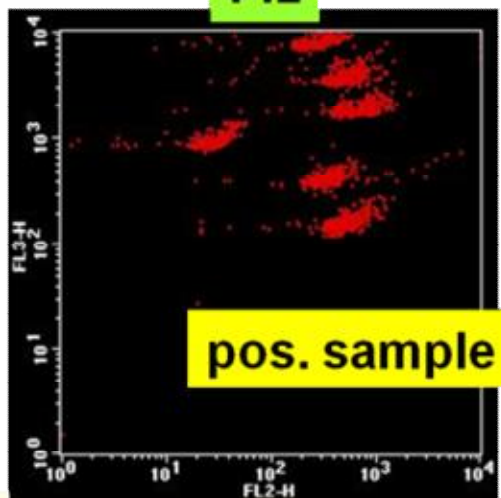
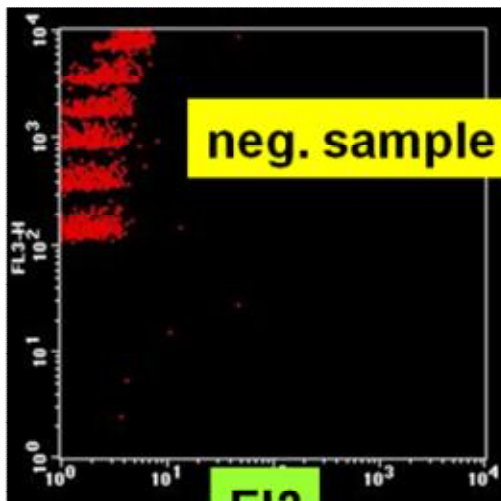


"Sandwich" assay

# Flow sitometri

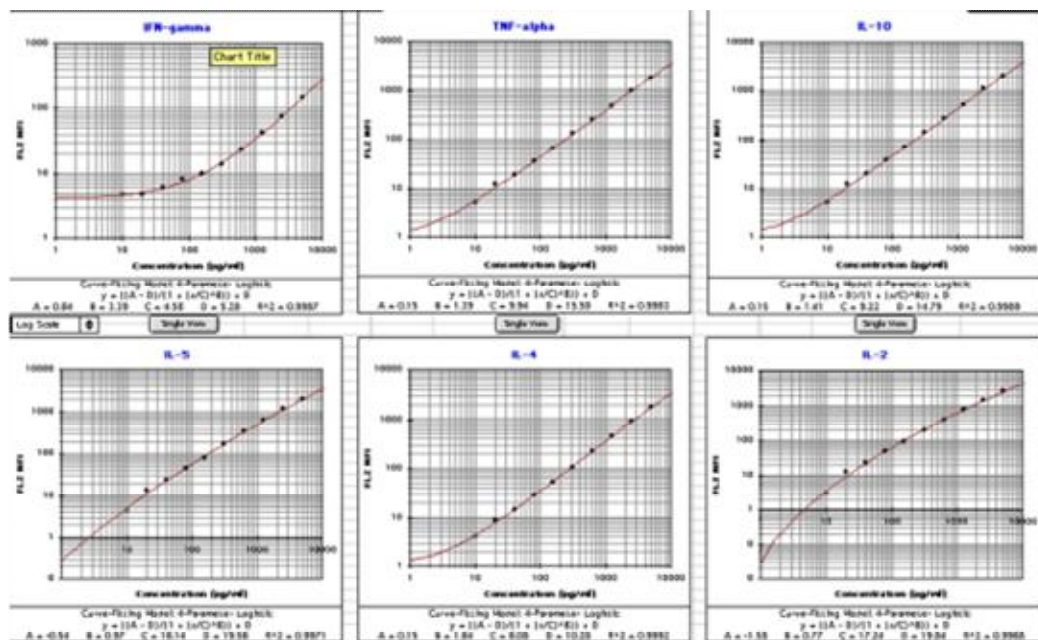


# Flow sitometri

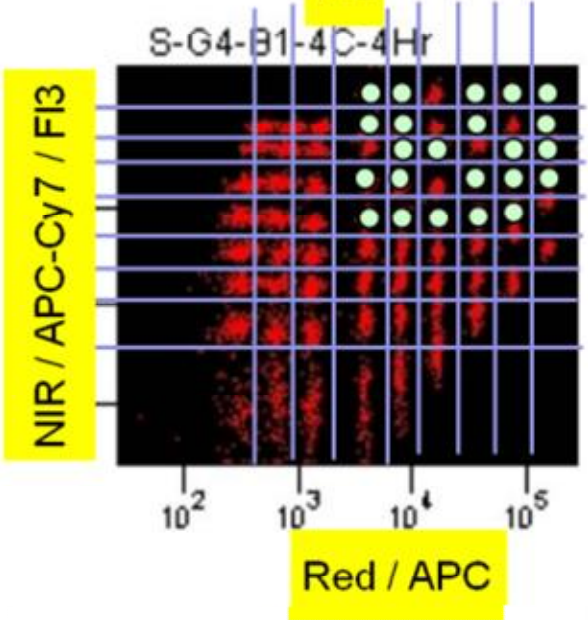
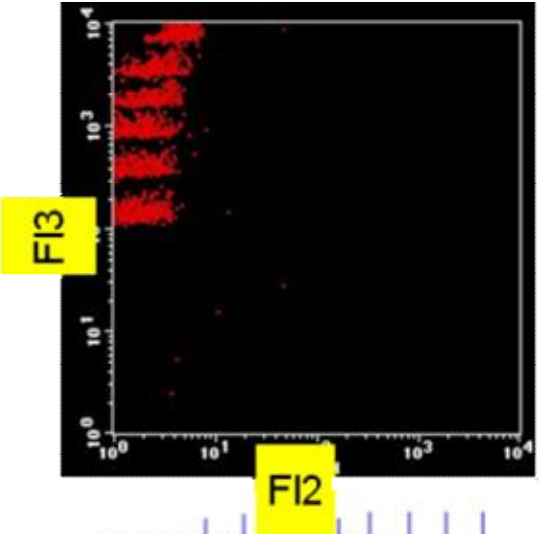
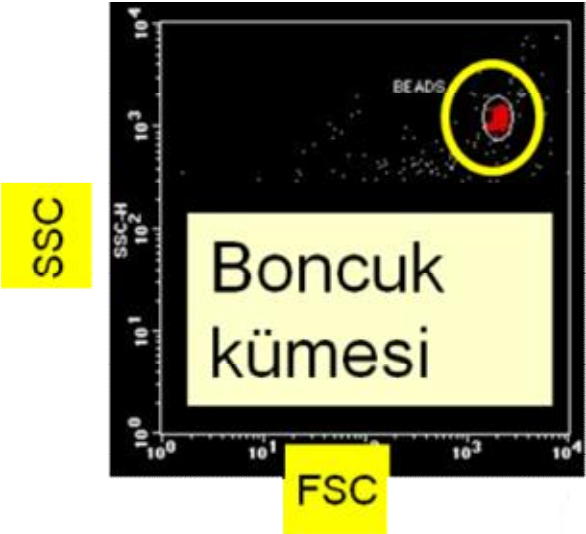


concentration cytokine

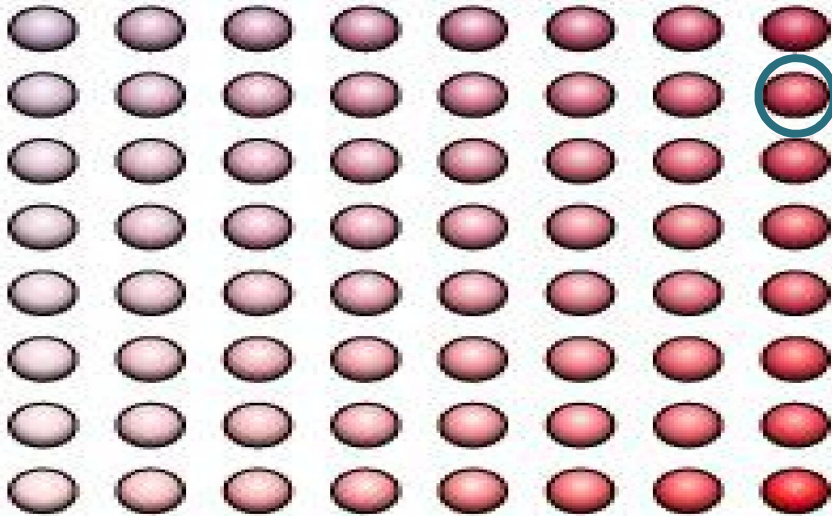
## standard curves



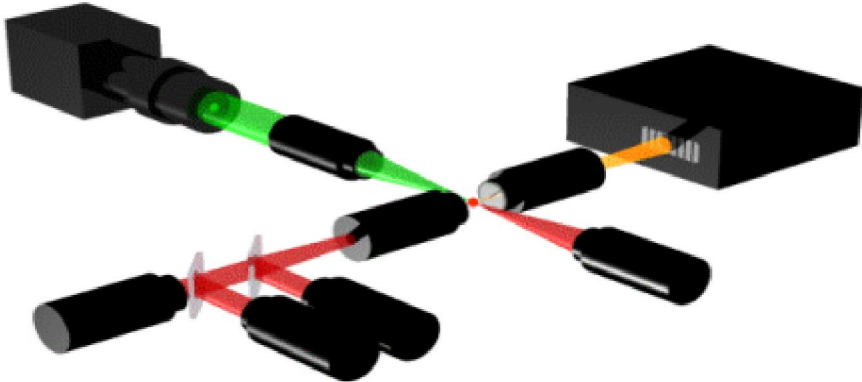
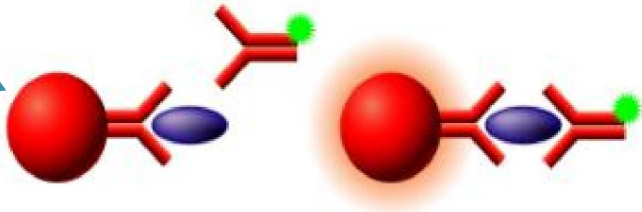
# Flow sitometri



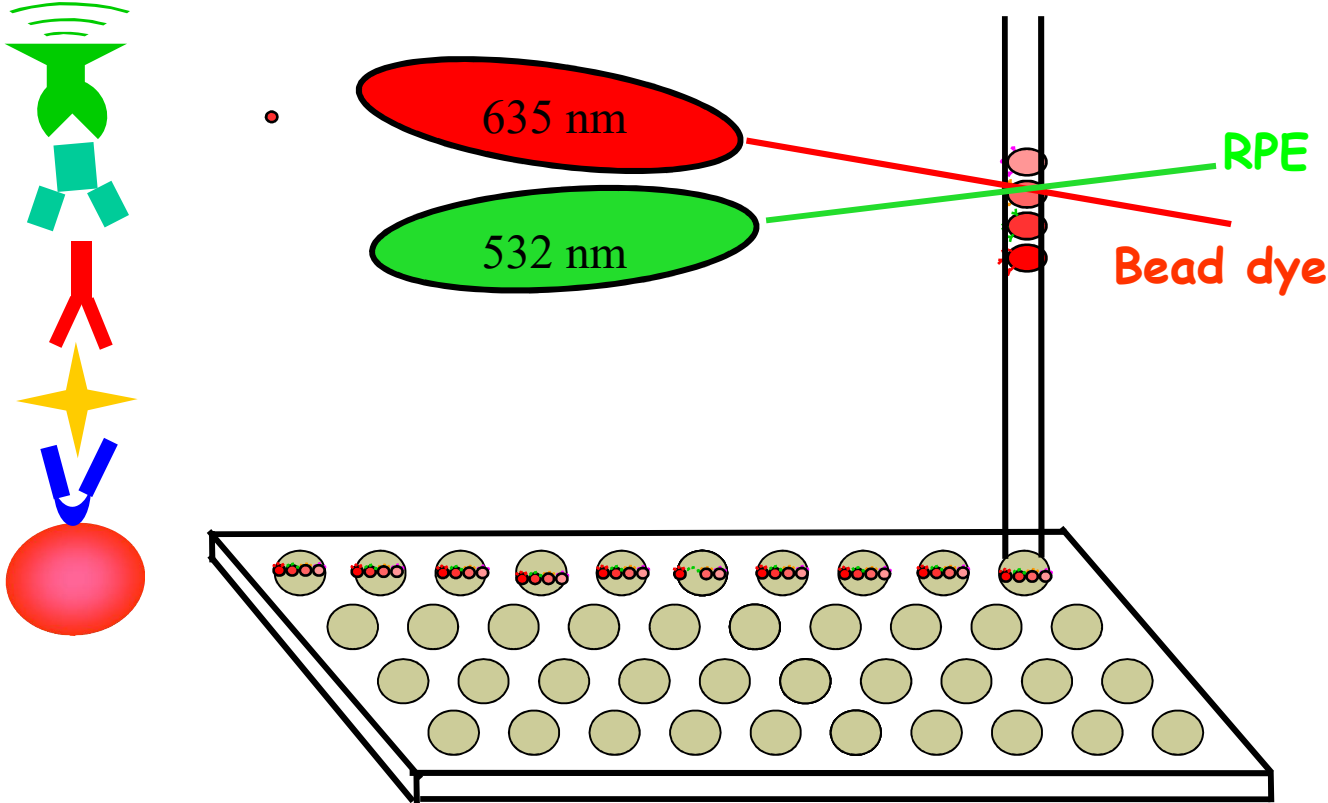
# Luminex



Immunoassay



# Luminex



Luminex 200, Luminex Corp.

