

Measuring Protein Concentration:

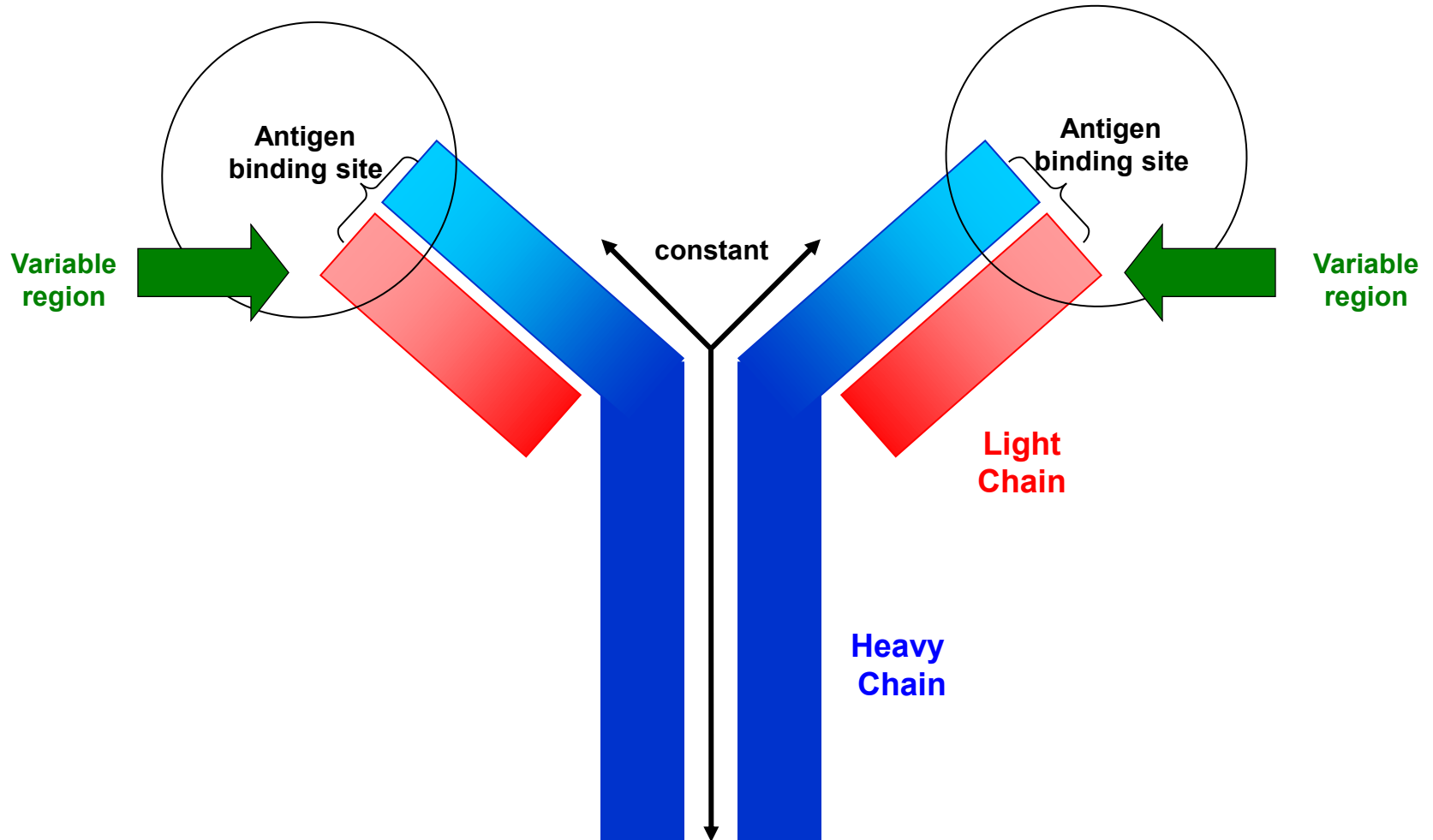
Enzyme-Linked Immunosorbent Assay

ELISA

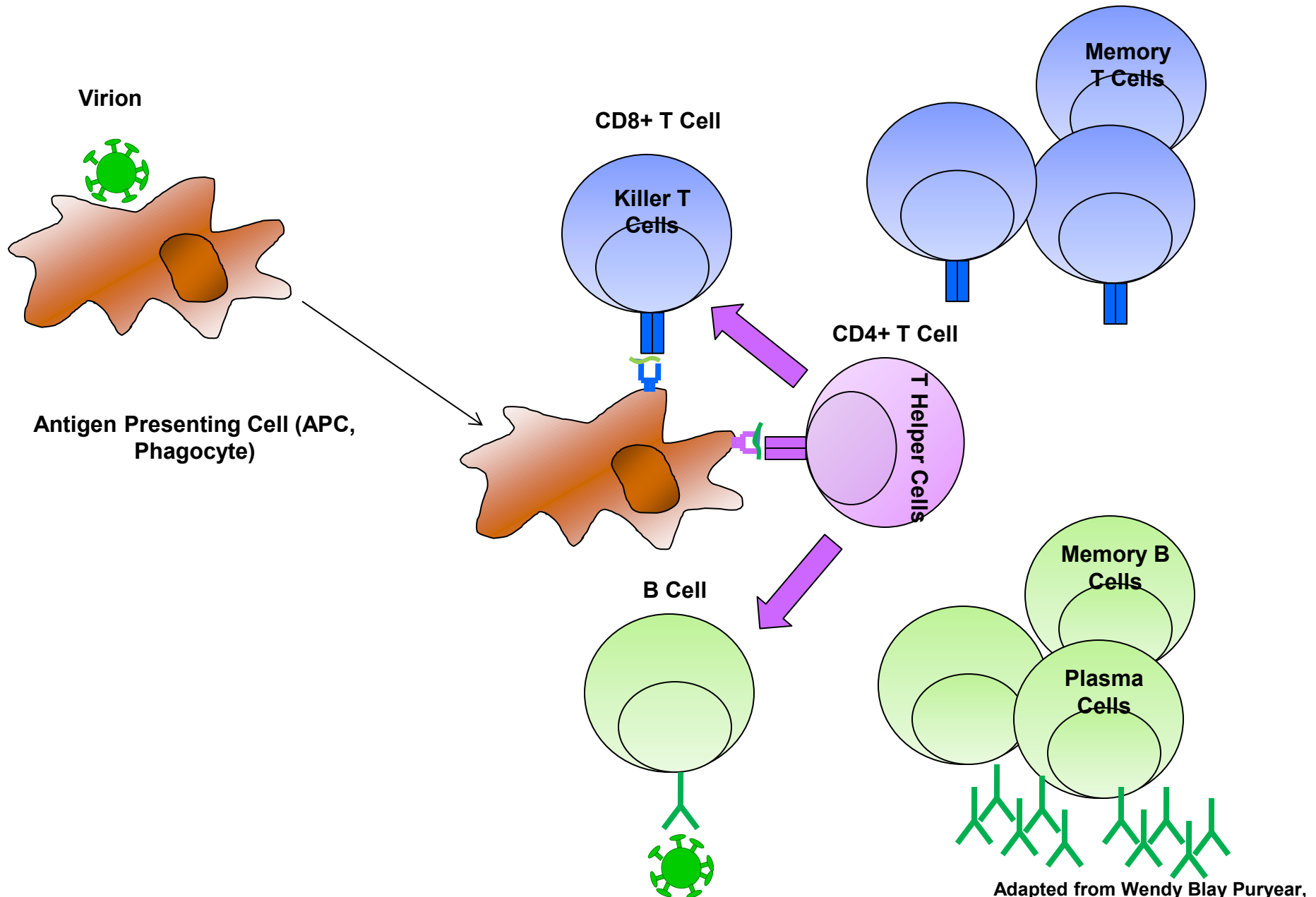
Biotechnology and Cancer
2019

What are Antibodies?

Antibodies

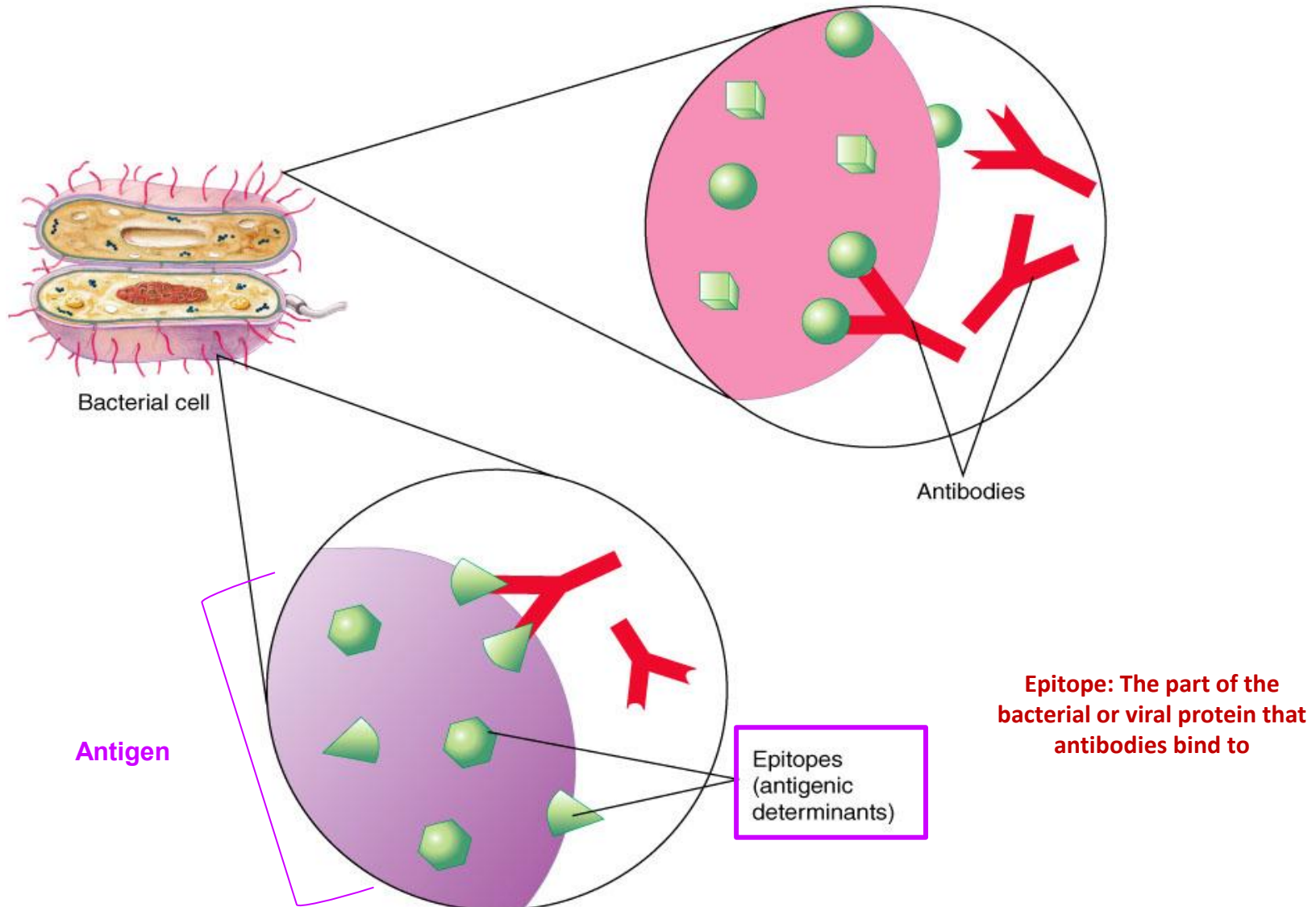


Immune Responses to Infection

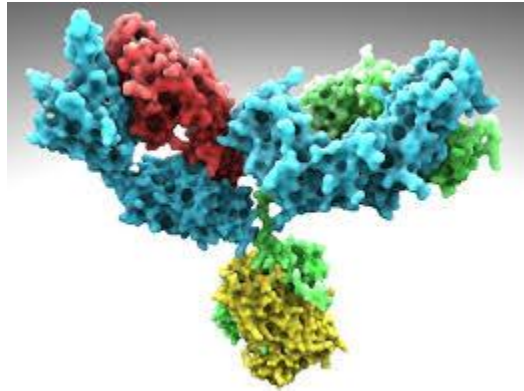


Antibodies Bind to Proteins

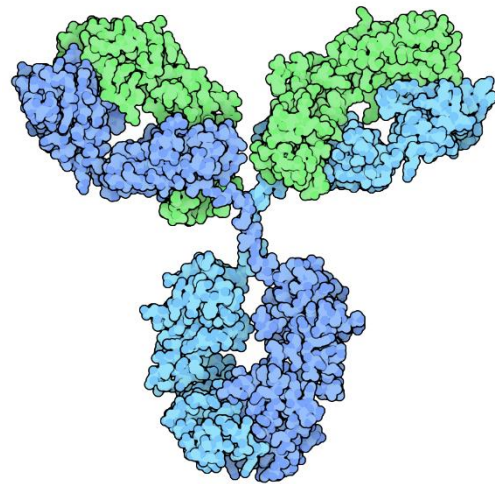
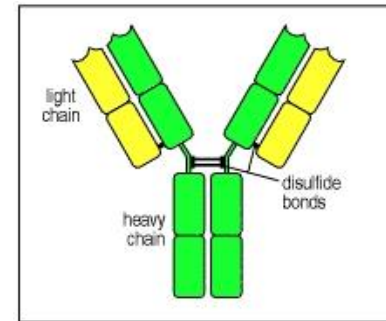
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What do Antibodies Look Like?



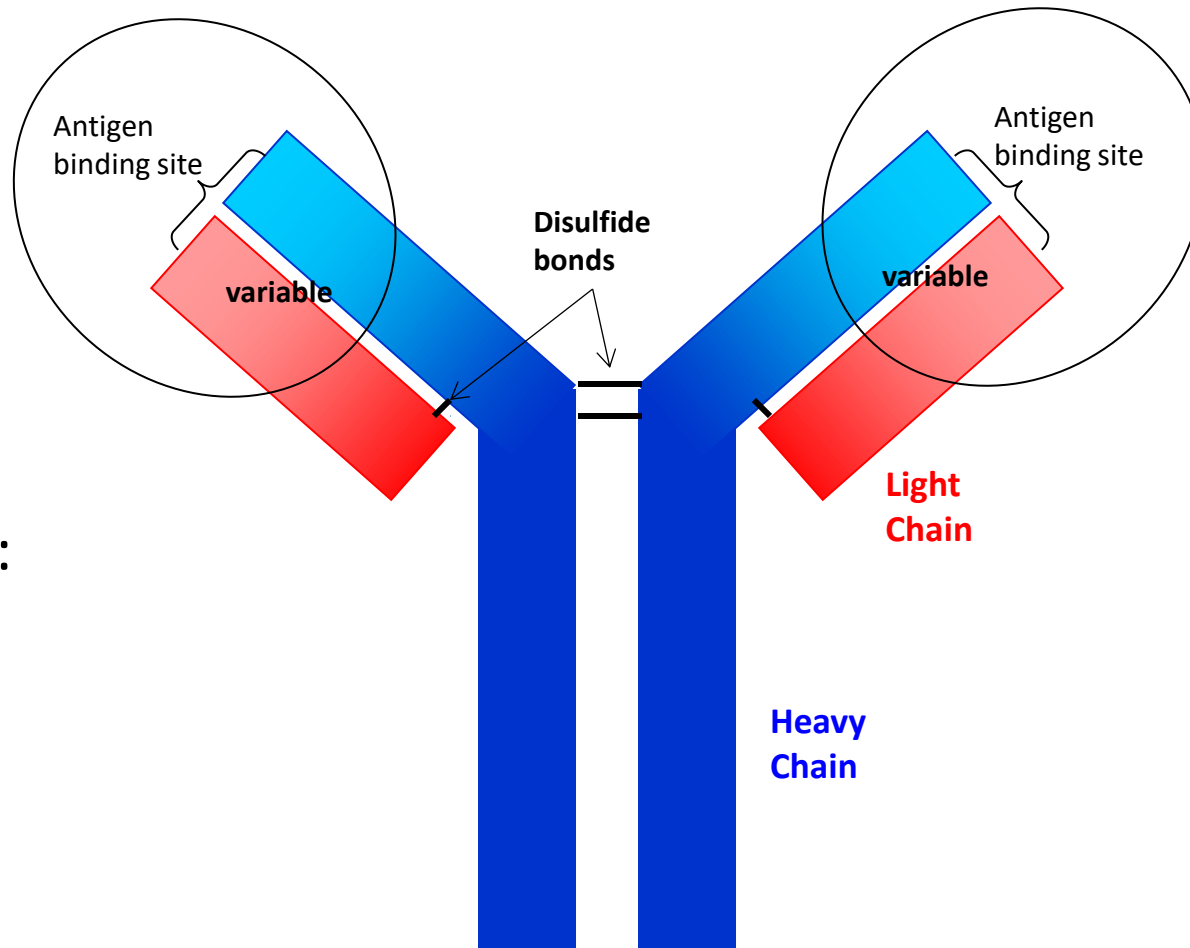
Y



There are many kinds of antibodies, also called **Immunoglobins ("Ig")**.

The most common is called **"IgG."**

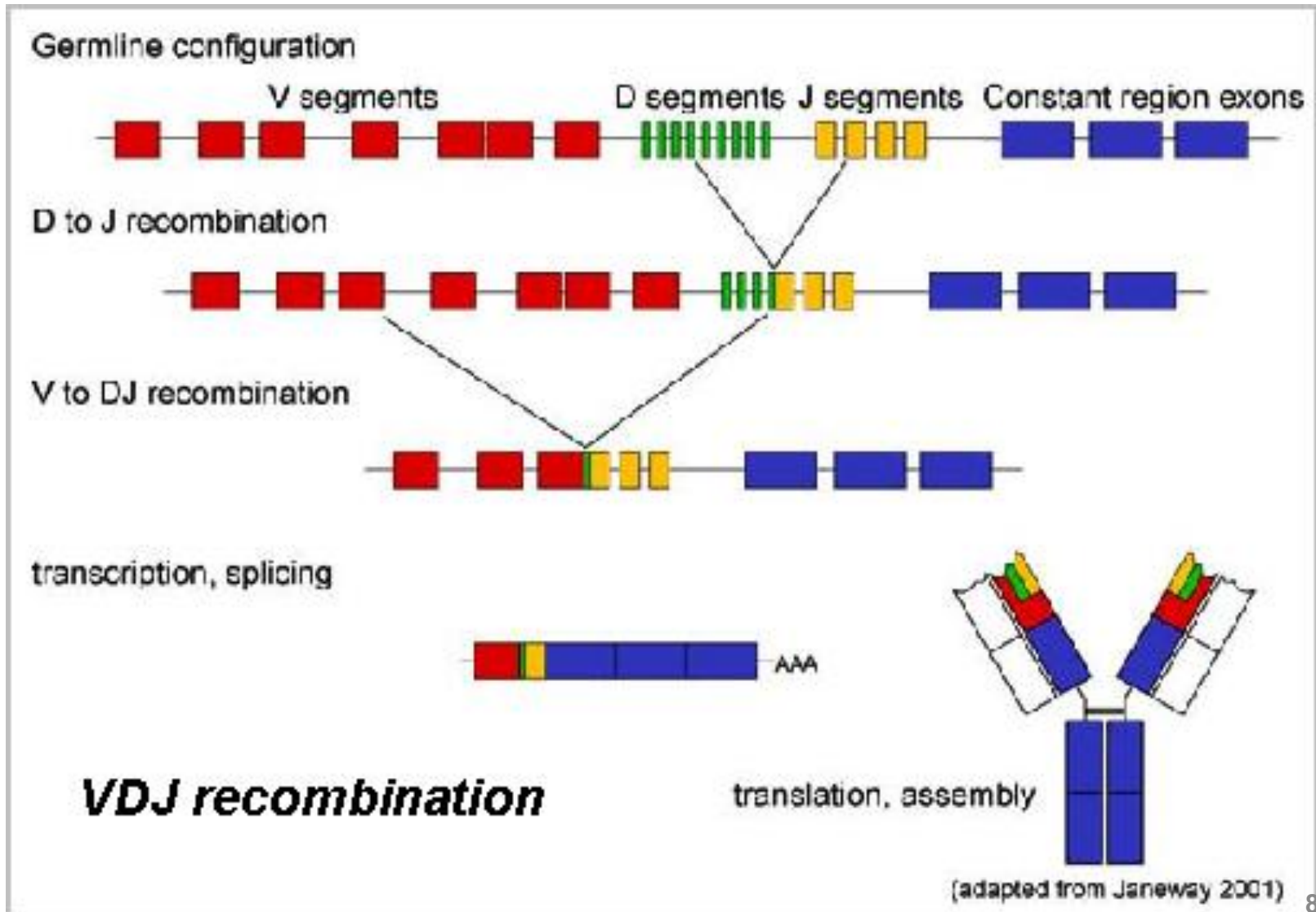
How do 2 genes create antibodies that can bind to any foreign object?



Isotypes:

- IgA
- IgD
- IgG
- IgE
- IgM

Antibody Diversity is Achieved via Genetic Recombination



The Human Genome Contains Multiple Heavy and Light Chain Gene Segments

Number of functional gene segments in human immunoglobulin loci			
Segment	Light chains		Heavy chain
	κ	λ	H
Variable (V)	40	30	40
Diversity (D)	0	0	25
Joining (J)	5	4	6

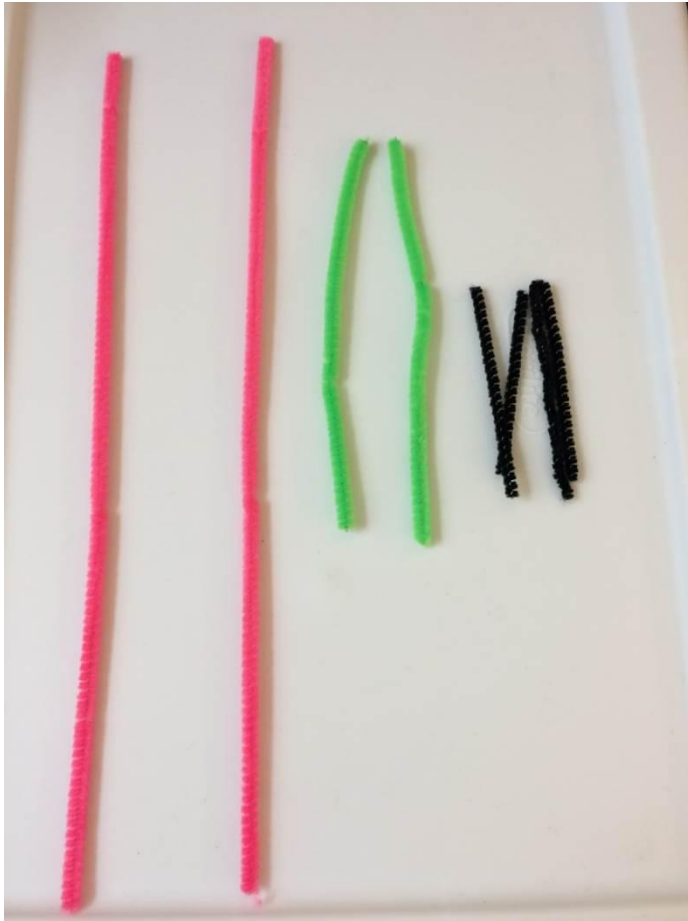
Figure 4-3 Immunobiology, 7ed. (© Garland Science 2008)

Antibody Model Activity



FRED HUTCH
Science
Education
Partnership

Gather Your Materials



- 2 long chenille stems of the same color = **2 heavy chains**
- 2 short chenille stems of the same color = **2 light chains**
- 4 small black chenille stem pieces = **disulfide bonds**

Disulfide Bonds on Heavy Chains



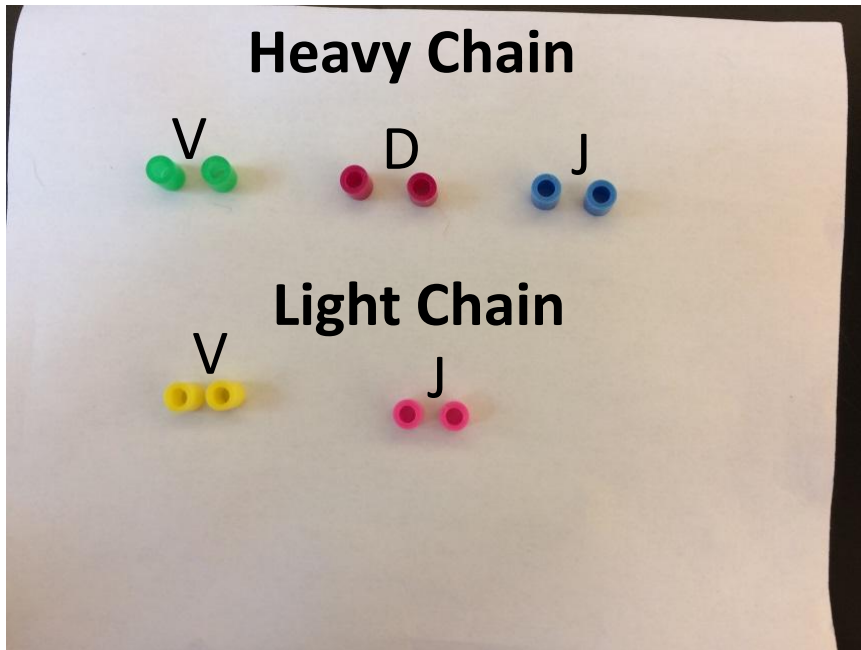
- Use two black 'disulfide bonds' to attach the two heavy chains to each other
- Bend the two 'arms' of the antibody model apart

Disulfide Bonds on Light Chains



- Attach each light chain to each heavy chain using 1 disulfide bond each

Increasing Antibody Diversity: V(D)J Regions



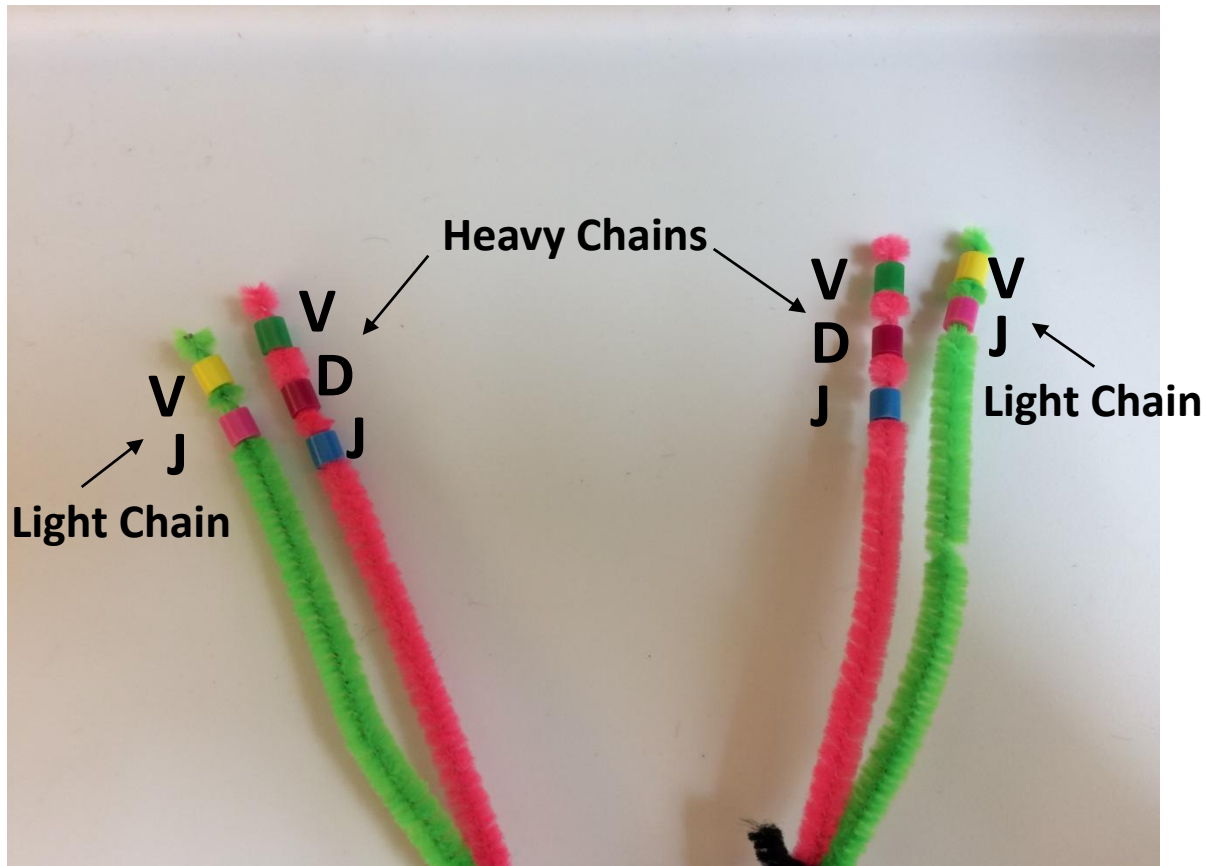
- **Heavy Chains:**

- Choose 2 “V” beads
- Choose 2 “D” beads
- Choose 2 “J” beads

- **Light Chains:**

- Choose 2 “V” beads
- Choose 2 “J” beads
- *Light chains don't have D regions*

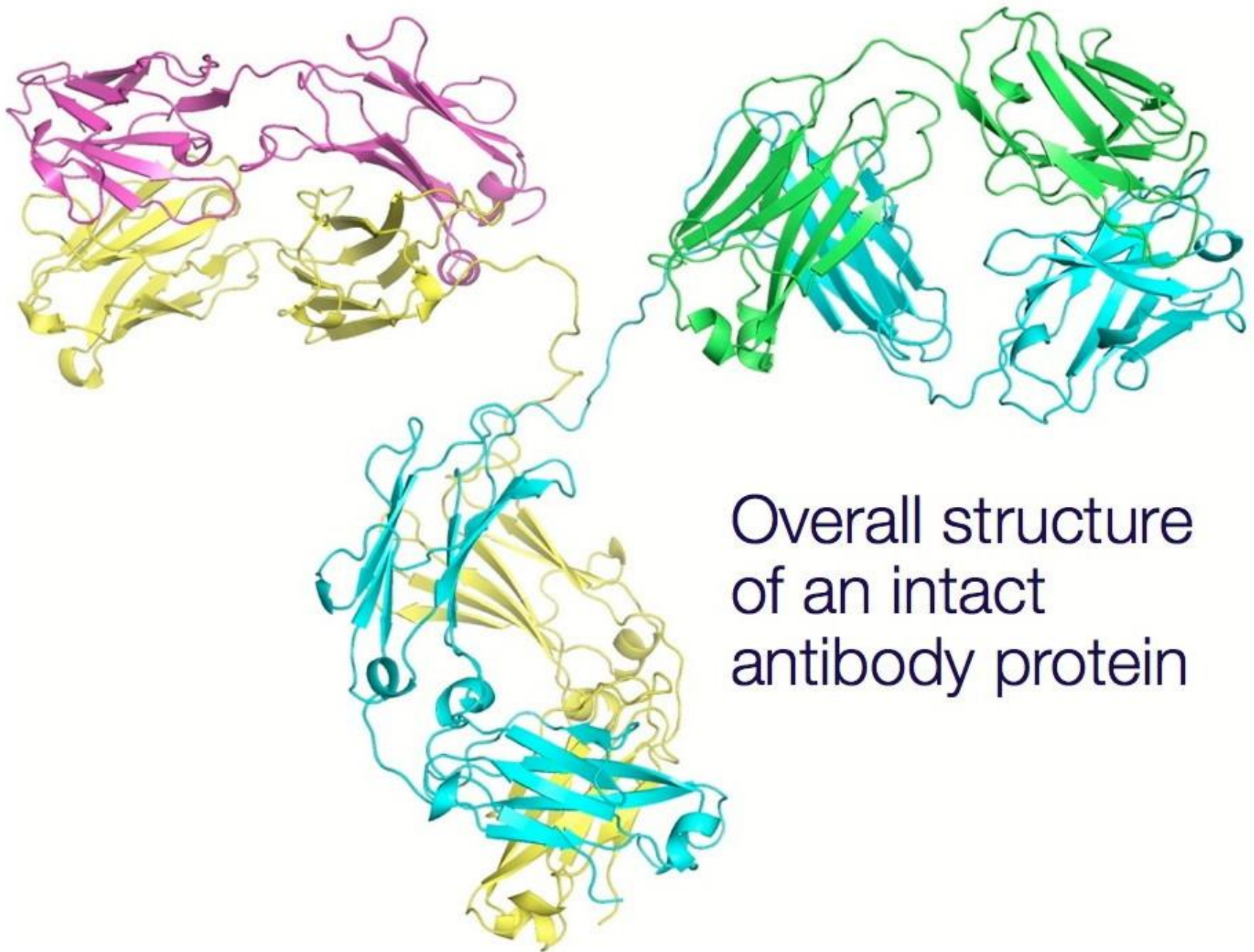
Increasing Antibody Diversity: V(D)J Regions



- Place the 3 V, D and J beads on each heavy chain
- Place the 2 V and J beads on each light chain

Final Antibody Structure: Each One is Different!





Overall structure
of an intact
antibody protein

Antibodies Can be Used as Research Tools

- Stimulate B cells with a foreign protein of interest
- Immortalize B cell to grow in culture
- Produce large quantities of antibody to protein of interest
- Label antibody with fluorescent molecule or other type of detectable marker
- Use labeled antibody to bind to and detect protein of interest:
 - on cells
 - in an assay

National Cancer Institute (NCI) Definition of a ***Biomarker***

- “A biological molecule found in blood, other body fluids, or tissues that is a sign of a normal or abnormal process, or of a condition or disease.”
- Biomarkers are used to differentiate between a person affected by a disease and a person without the disease.

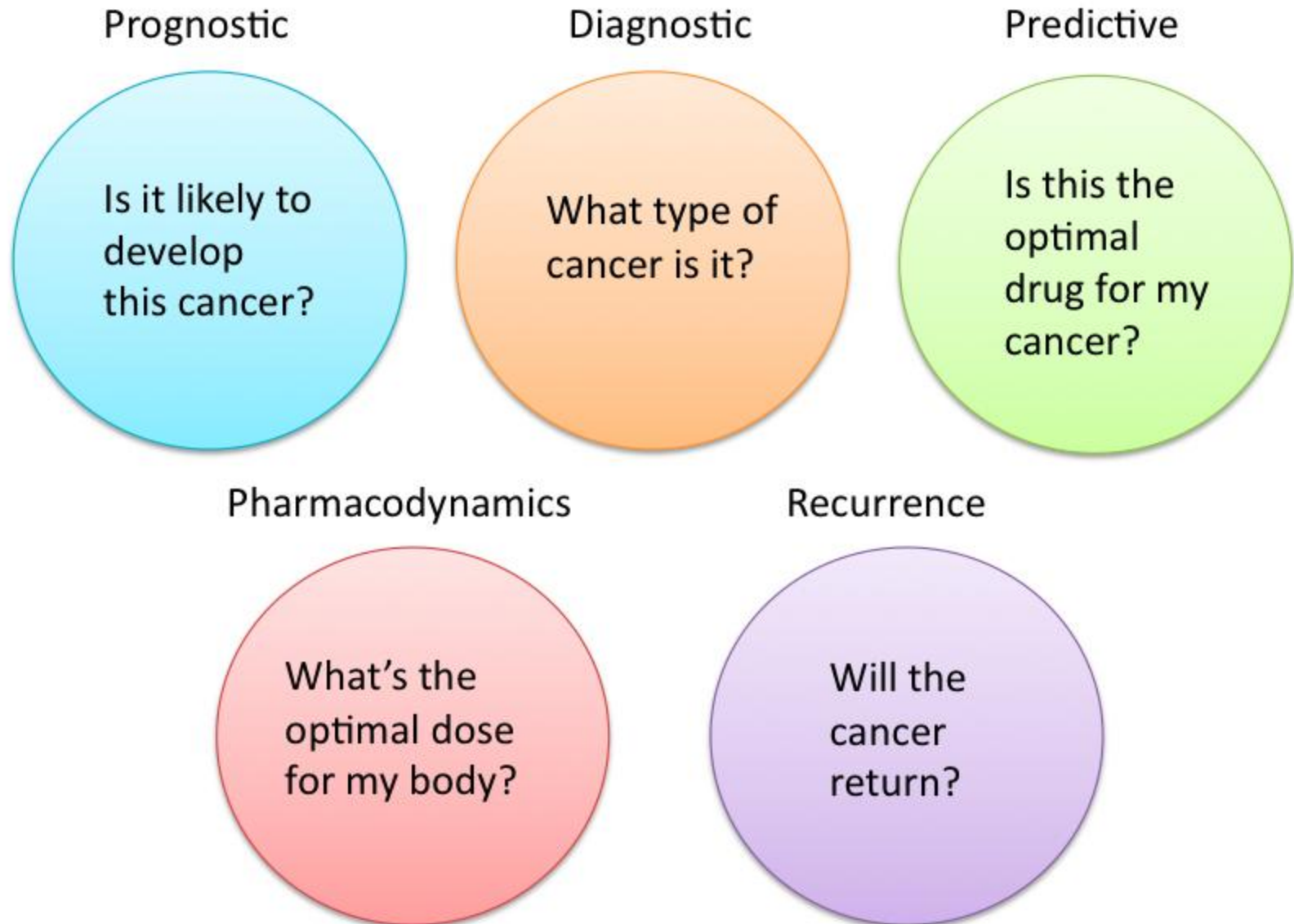
Types of Biomarkers

- Proteins (enzyme, receptor etc)
- Nucleic acids (microRNA, other non-coding RNA)
- Antibodies
- Peptides
- Changes in gene expression
- Changes in metabolic signature

Biomarker Detection

- Circulation:
 - Blood, Serum, Plasma
- Excretions or Secretions:
 - Stool, Urine, Sputum
- Tissue:
 - Requires biopsy or other imaging
- Genetic:
 - DNA or RNA isolated from patient tumor cells

Questions that can be answered by cancer biomarkers



Examples Uses of Biomarkers

- Determination of a patient's *risk* of developing cancer
 - BRCA1, BRCA2 mutations (breast cancer, ovarian cancer)
- *Diagnosis* of a patient's cancer – determination of the presence of disease
 - Elevated levels of CEA (lung cancer)
 - Genetic translocations/Philadelphia chromosome (AML)
- Determine *prognosis* -- can the disease be treated successfully
 - Elevated levels of metalloproteinase inhibitor 1 (TIMP 1) – a marker associated with more aggressive forms of multiple myeloma
- How should the cancer be *treated*
 - Kras mutations and anti-EGFR treatment (colorectal cancer)

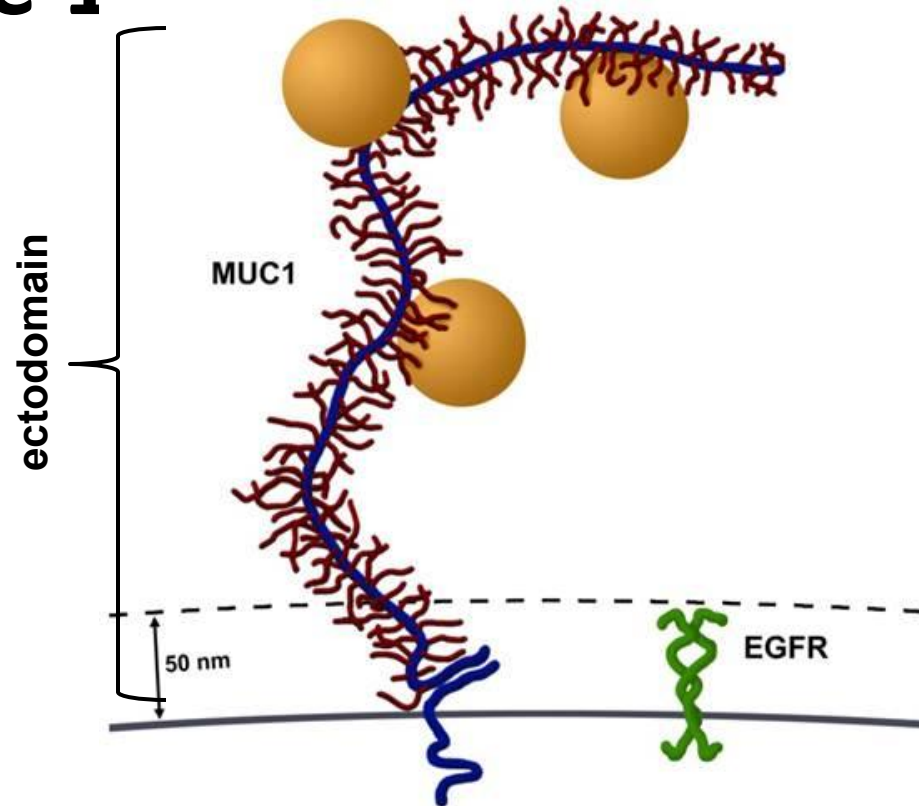
**Biomarkers found in bodily fluids are
the easiest to sample**

**They are often detected using the
ELISA test**

**Breast Cancer Antigen CA 27.29 is
found on the MUC1 protein.**

MUC-1

- Found on the surface of cells
- Altered form found in breast cancer cells
- Part of MUC-1, called CA 27.29, is shed from the cell surface during *metastasis*



MUC-1 Biomarker

CA 27.29



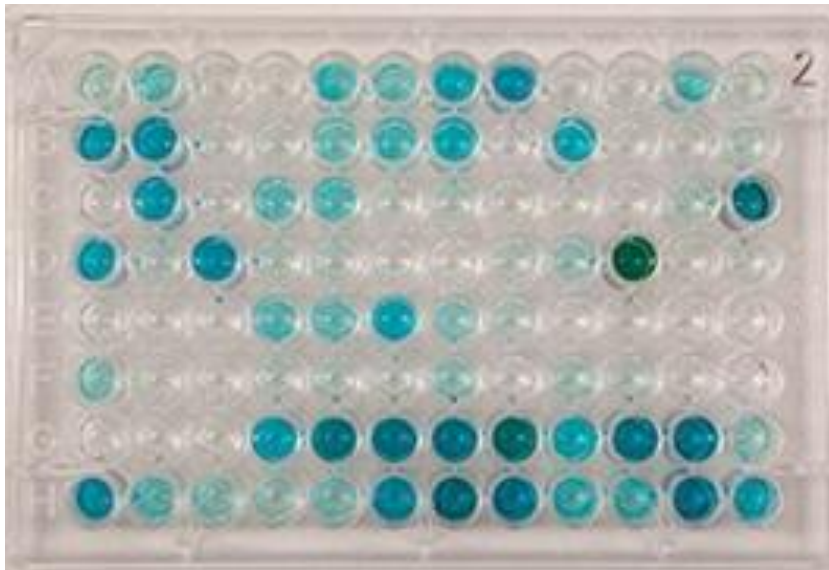
MUC1: A Target Molecule for Cancer Therapy

R Singh and D Bandyopadhyay, Cancer Biol and Therapy (2007) 6: 481-486

ELISA Activity

ELISA: Analyzing Many Samples at One Time

ELISA Plate During Development



ELISA Plate After Development is Stopped



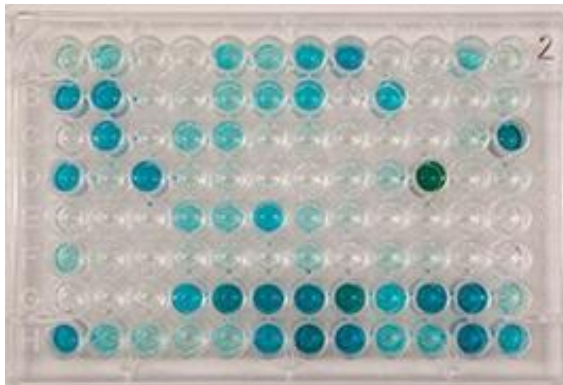
Horseradish Peroxidase (HRP)



PDB ID 1W4W

- Found in the roots of horseradish plants
- Converts a substrate (3,3',5,5'-Tetramethylbenzidine or TMB) from a colorless solution to a **blue** solution
- Because this is an enzymatic reaction, it will continue to completion unless “stopped” with acid (1M H_2SO_4)
- *Amount of color is a measure of amount of analyte initially present*

ELISA Plate During Development



ELISA Plate After Development is Stopped



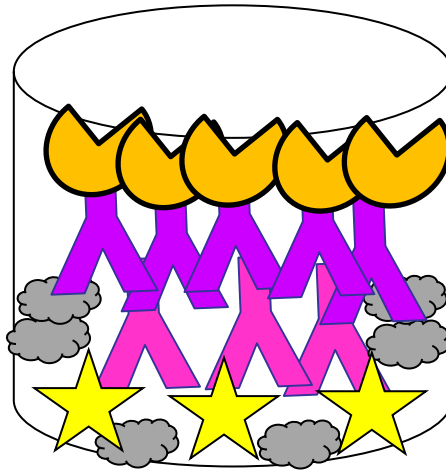
Basic ELISA Steps:

ELISA Models

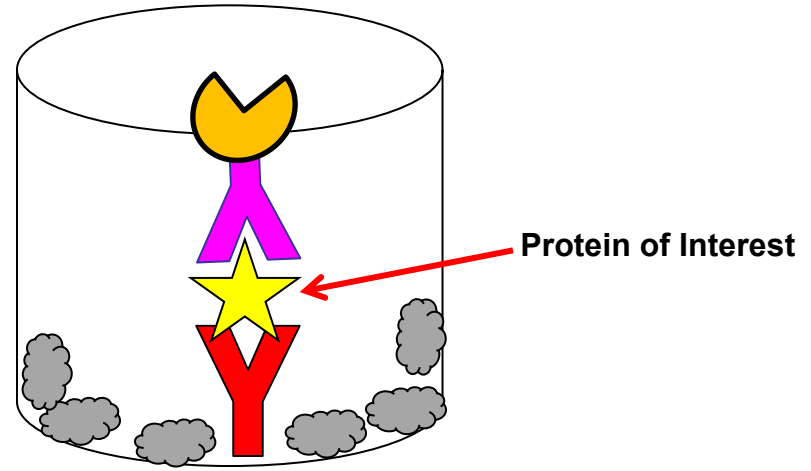
- **Coat** Plate with Antigen
- **Block** unbound space on plate to prevent non-specific binding
- Add patient **samples**, positive and negative controls
- **Wash** away unbound protein
- Add enzyme-conjugated **antibody**
- **Develop** by adding substrate (TMB) for enzyme (HRP)
- **Stop** the reaction
- **Analyze** data

Different Types of ELISA

Indirect



Sandwich



Sandwich ELISA:

Enzyme-Linked Secondary Antibody

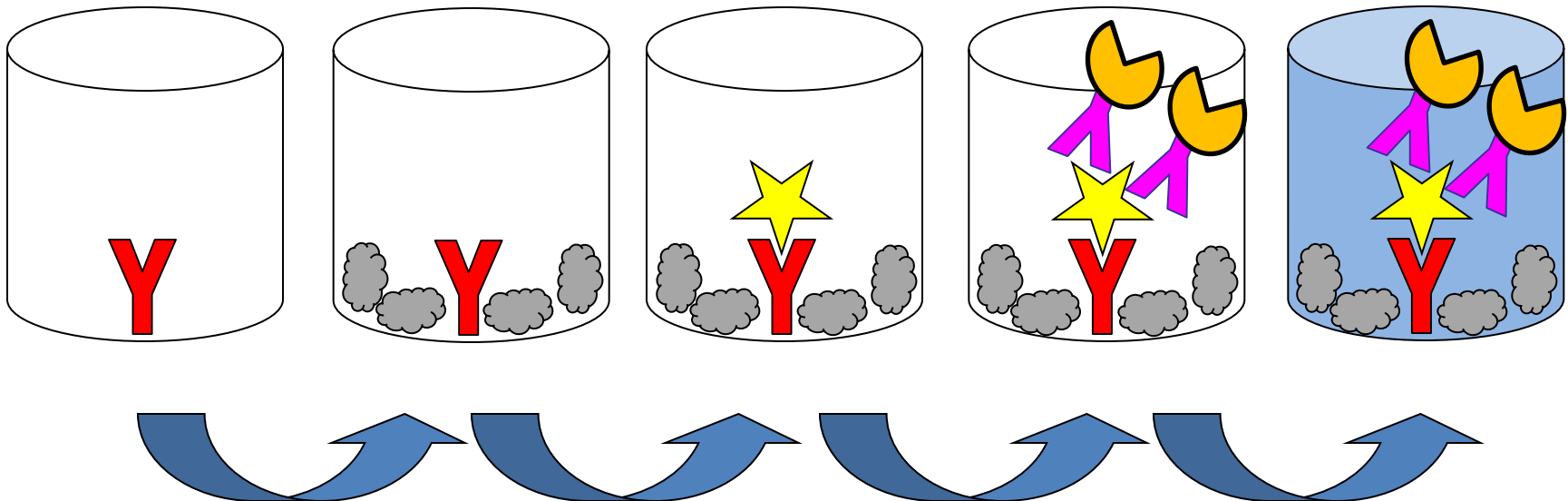
**Primary or
Capture/Coating
Antibody**
[specific for Ag]

Blocker

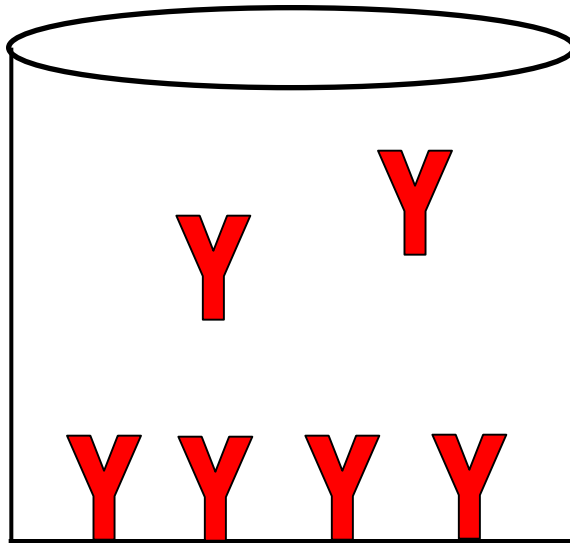
Antigen

**Secondary
Antibody**
[specific for Ag:
Conjugated to HRP]

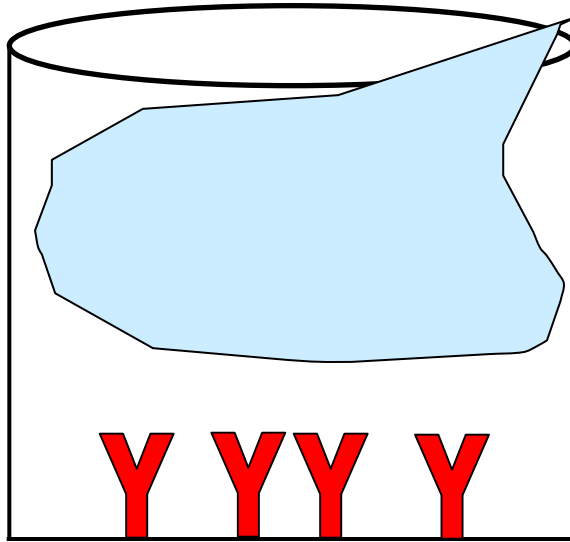
**Color
Substrate**



Coat the Bottom of the Well with Antibodies to the Protein of Interest



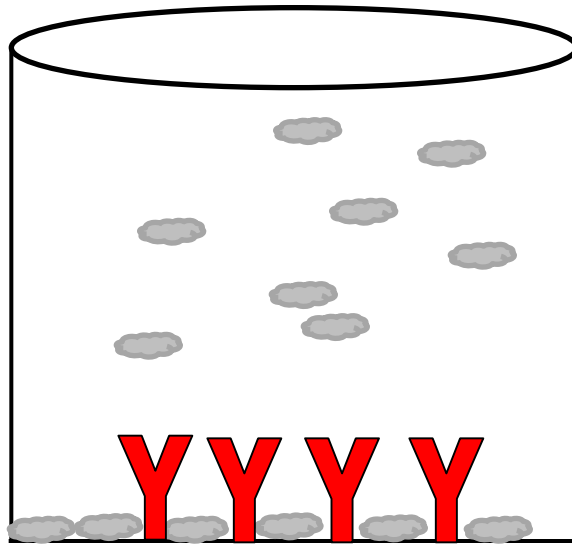
Wash Away Unbound Antibody



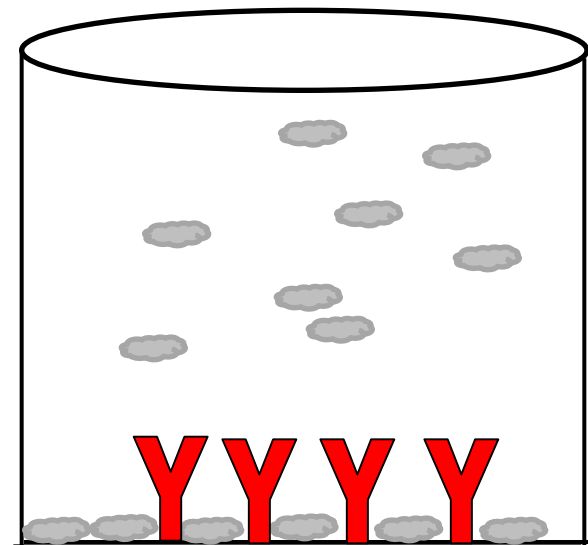
Add Blocking Solution

Milk Proteins Block Extra Spaces

Positive Control

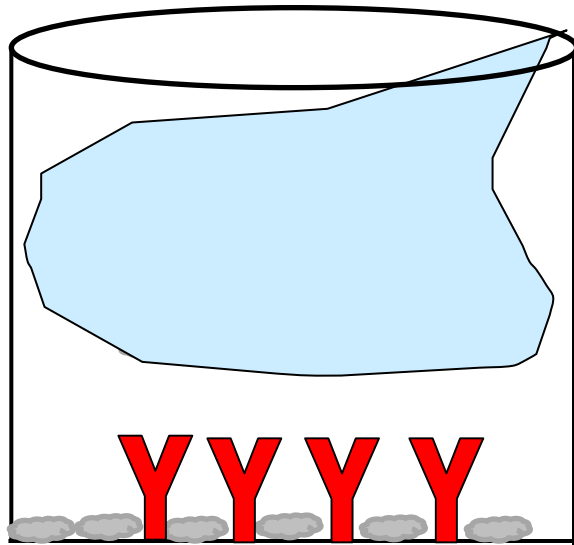


Negative Control

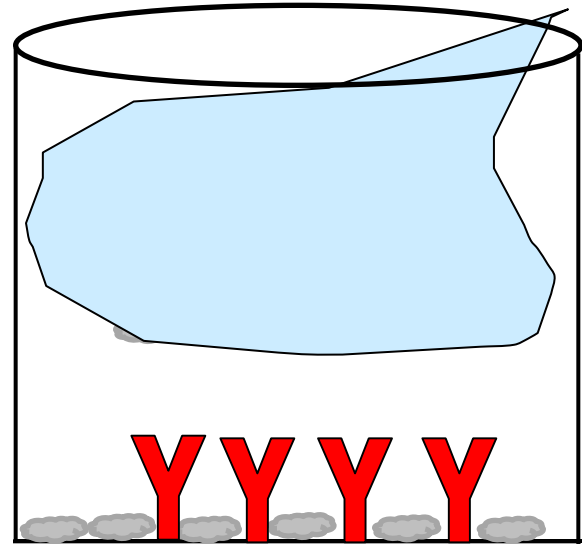


Wash Away Excess Blocking Solution

Positive Control

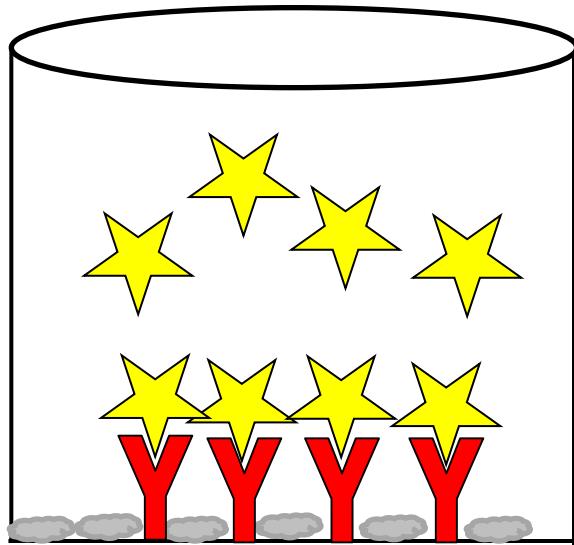


Negative Control



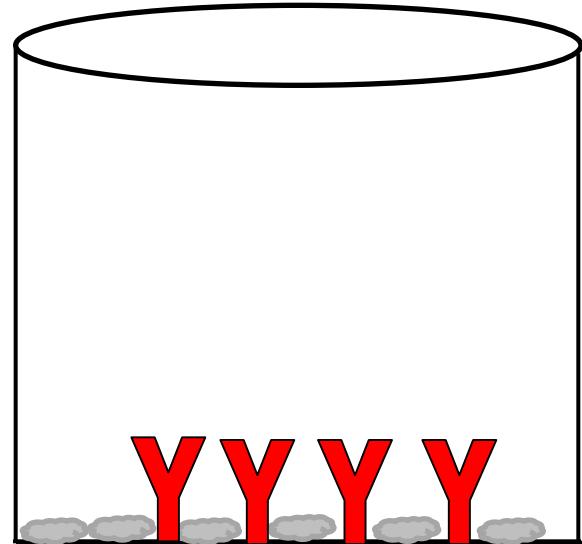
Positive and Negative Controls

Positive Control



Add Protein of Interest

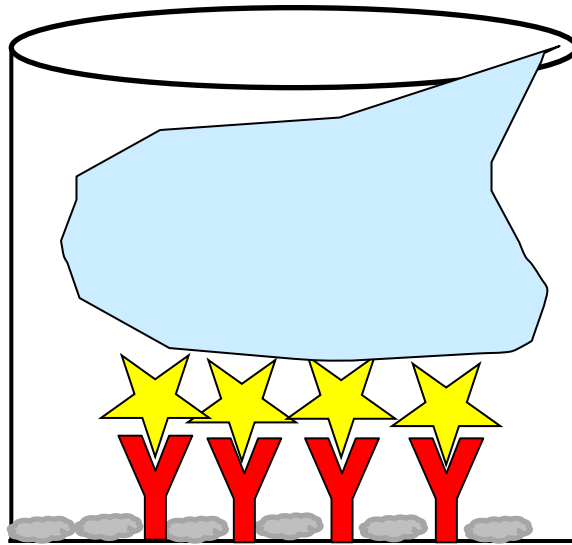
Negative Control



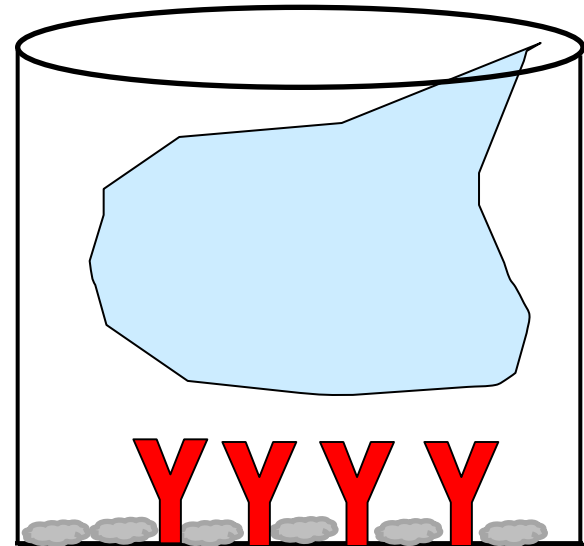
No Protein of Interest

Wash Away Unbound Protein of Interest

Positive Sample

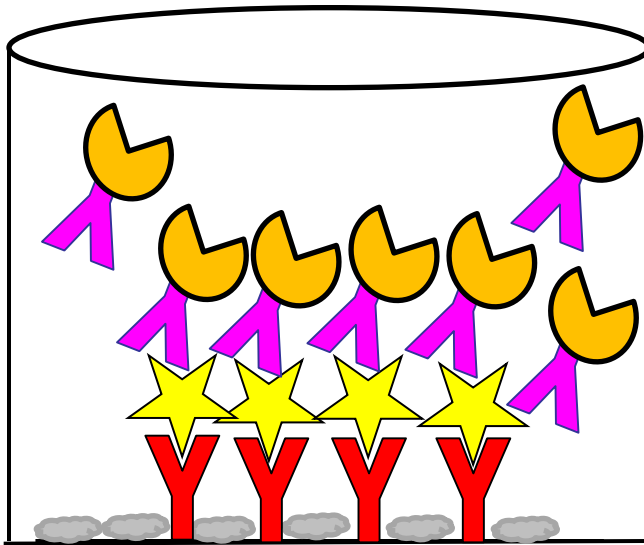


Negative Sample

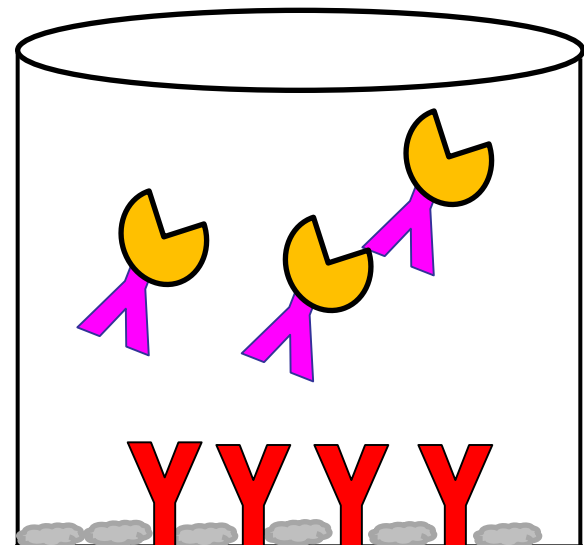


Add Secondary Antibody Linked to an Enzyme **Horse Radish Peroxidase (HRP)**

Positive Sample

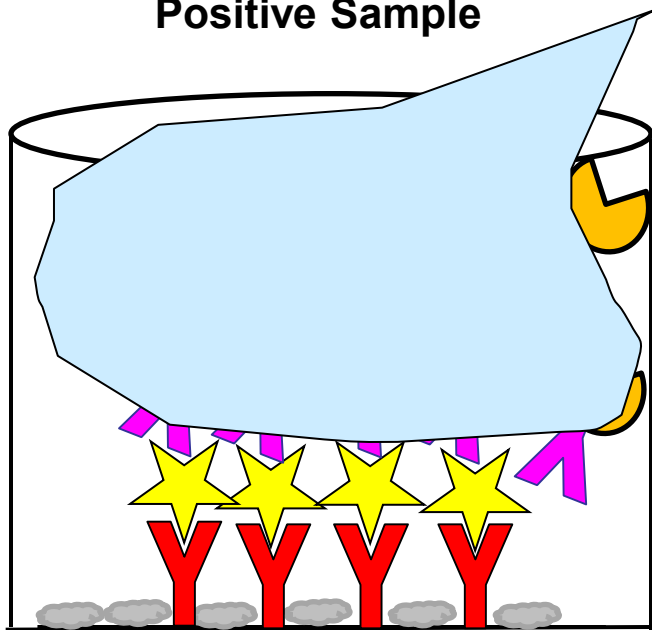


Negative Sample

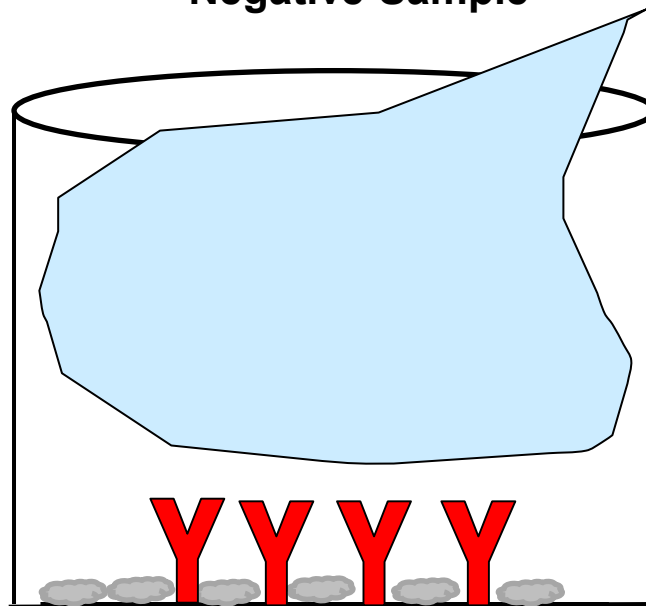


Wash Away Unbound Secondary Antibody

Positive Sample

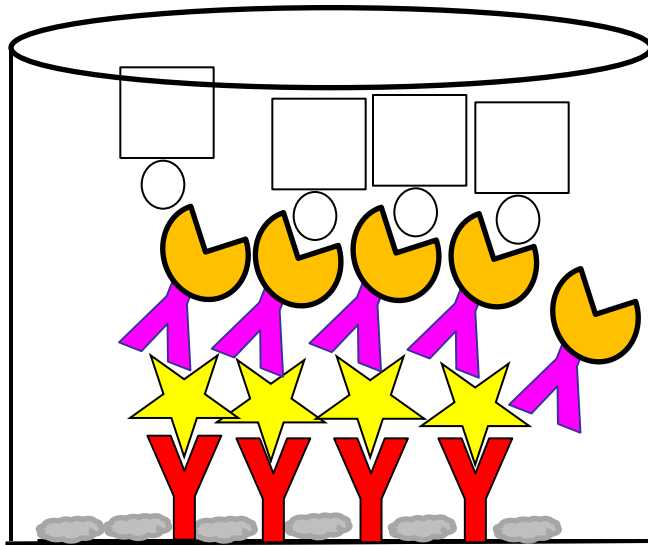


Negative Sample

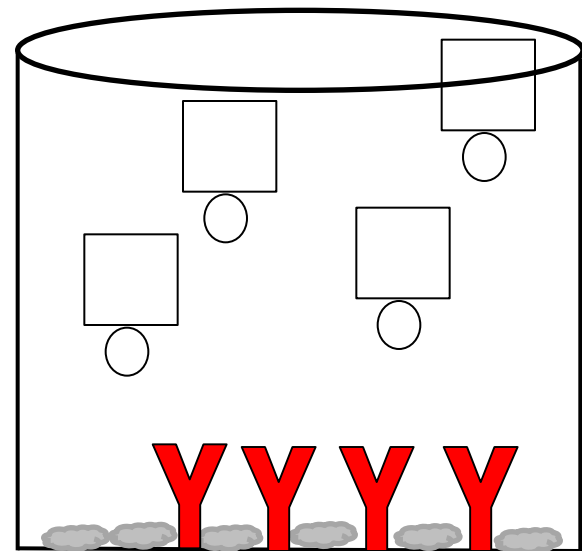


Add the HRP Enzyme Substrate: TMB

Positive Sample

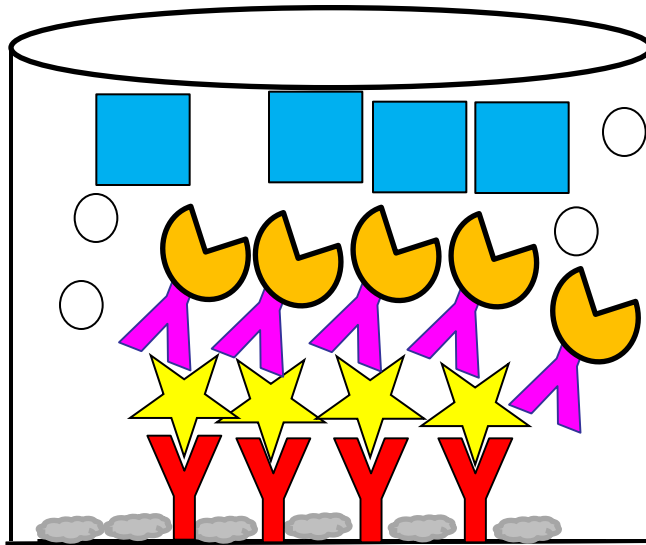


Negative Sample

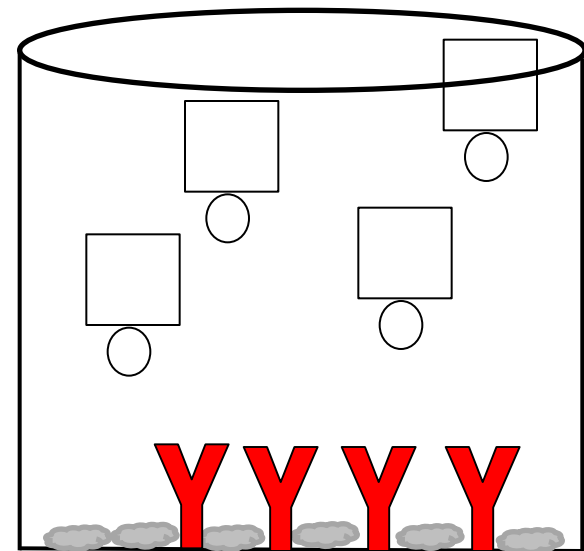


HRP Cleaves TMB Substrate: Product is Blue

Positive Sample



Negative Sample

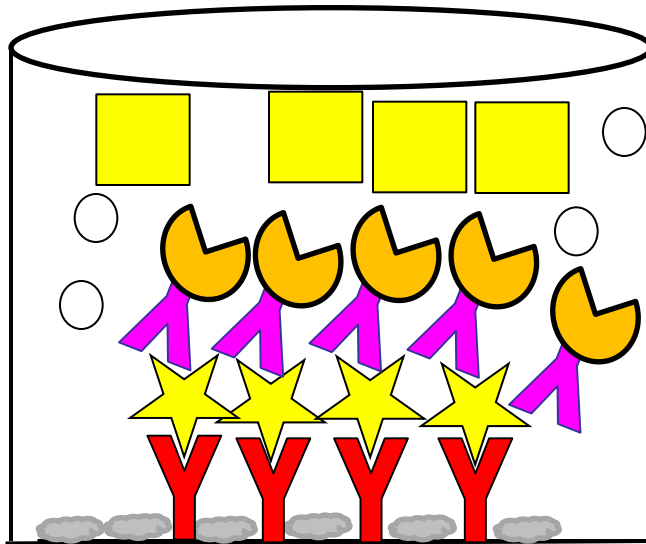


Wells with the protein of interest in them turn

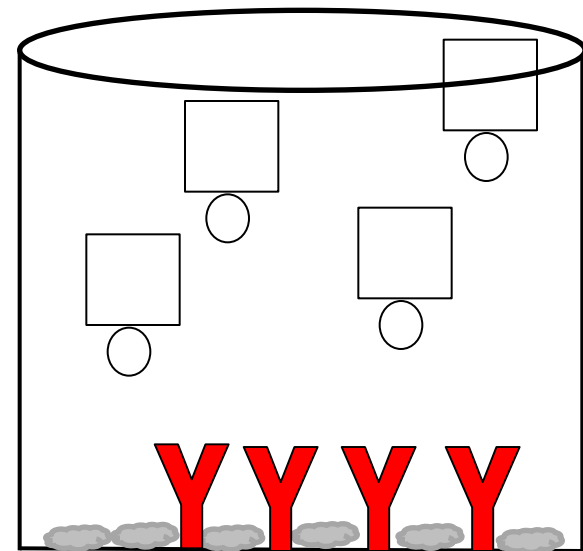
BLUE

The Stop Reagent Inactivates the HRP

Positive Sample

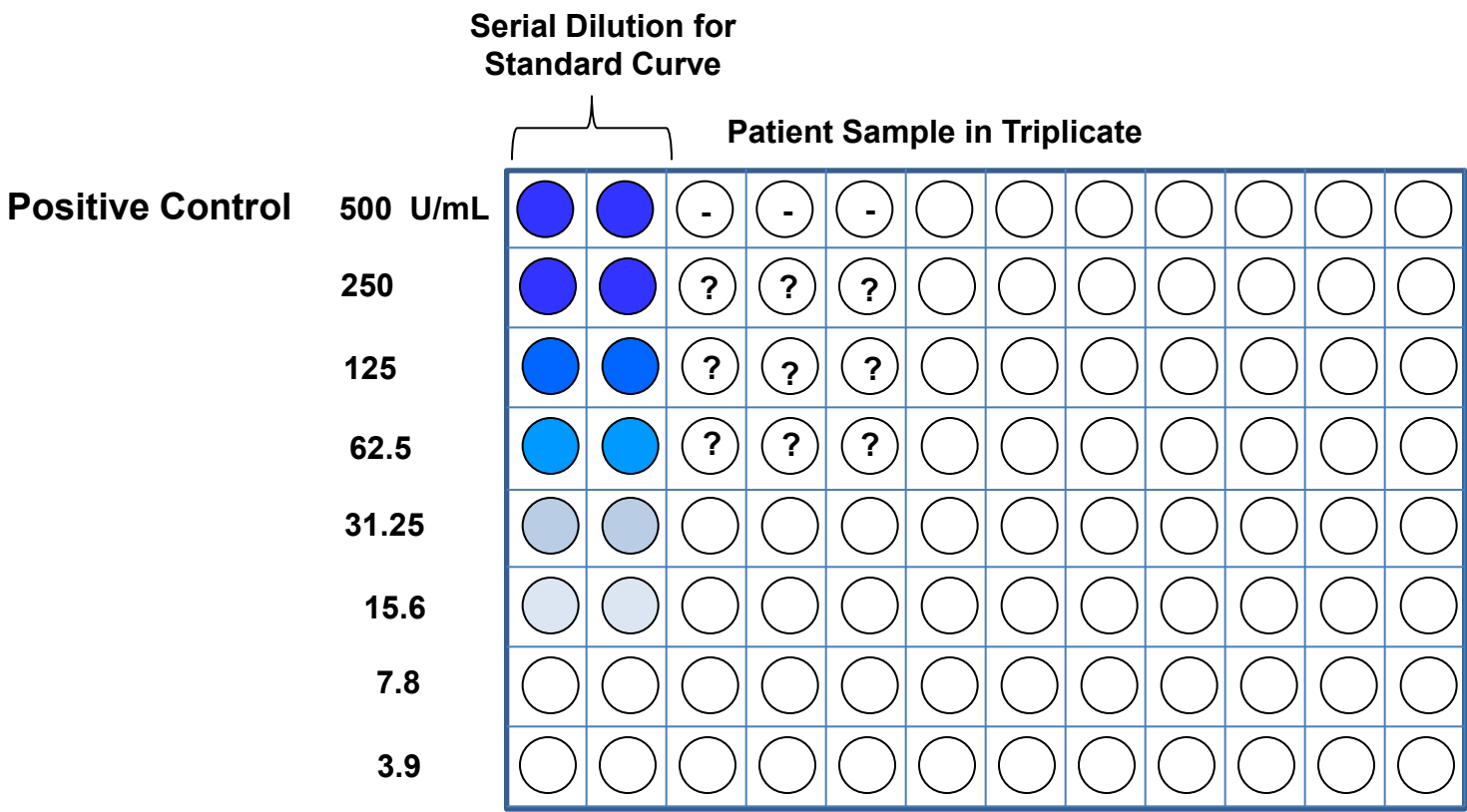


Negative Sample



Wells that have protein of interest in them
turn **YELLOW**

ELISA Plate Read Out With TMB Substrate



ELISA Plate Read Out

After Addition of Stop Solution

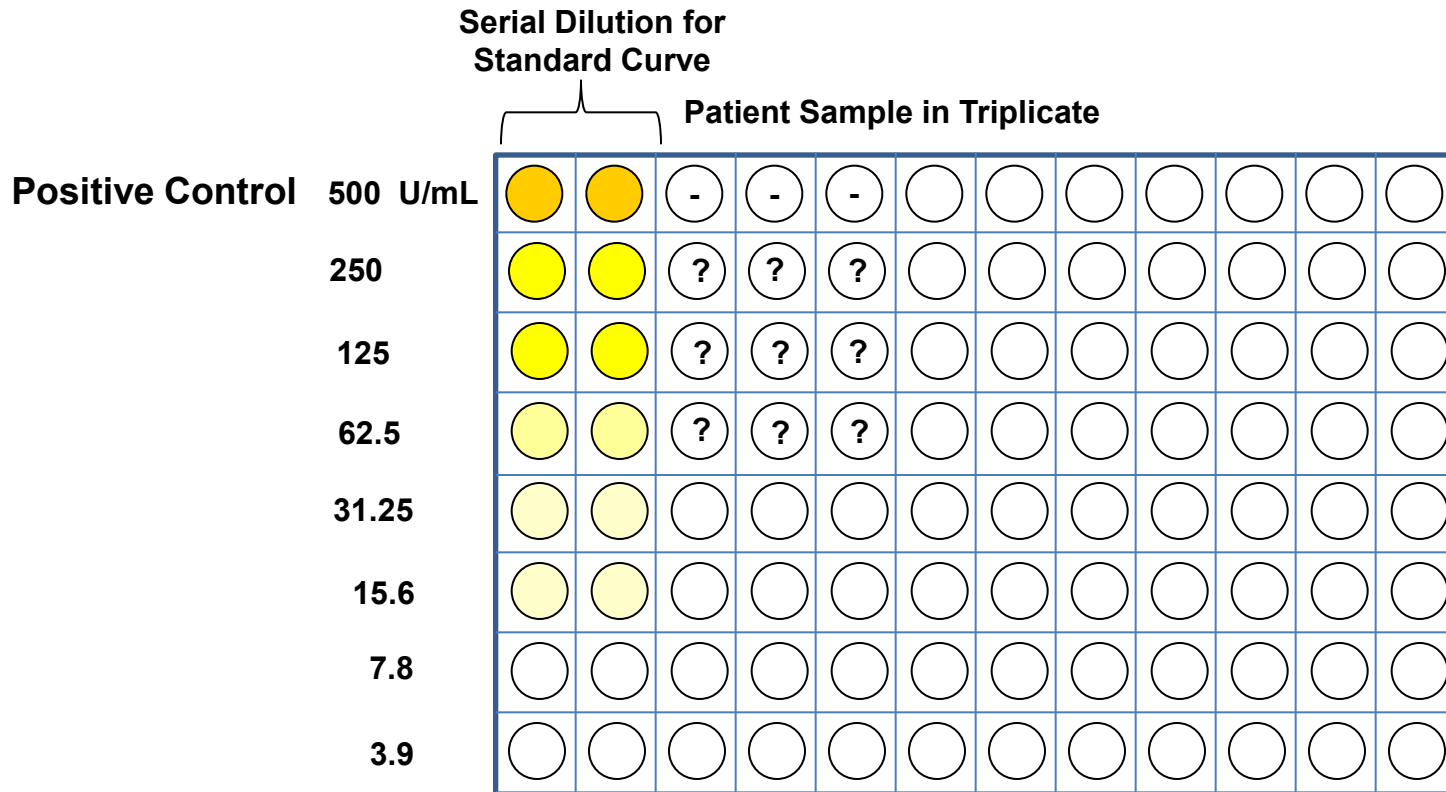


Plate Plan

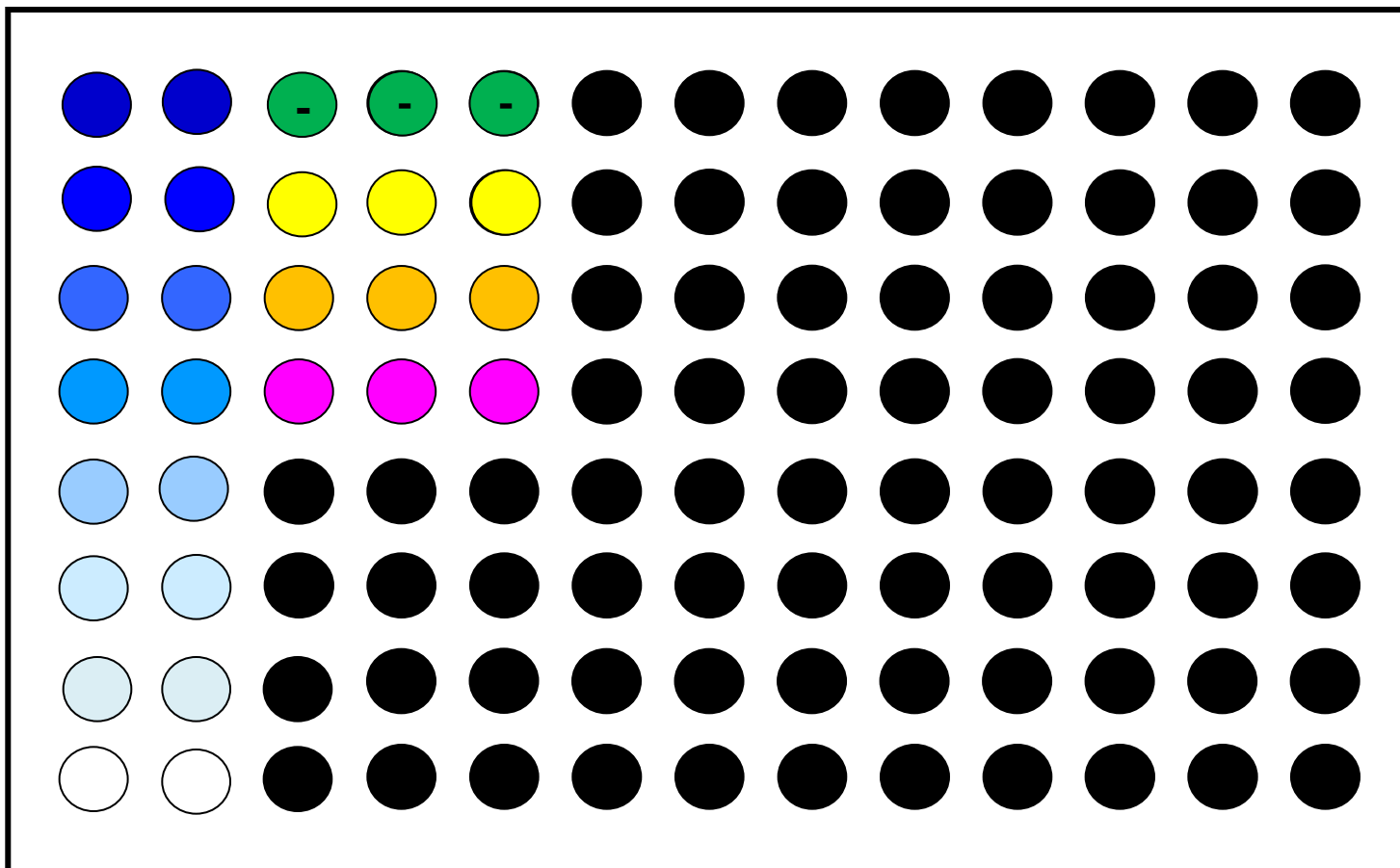
Standards

Negative Control in Triplicate

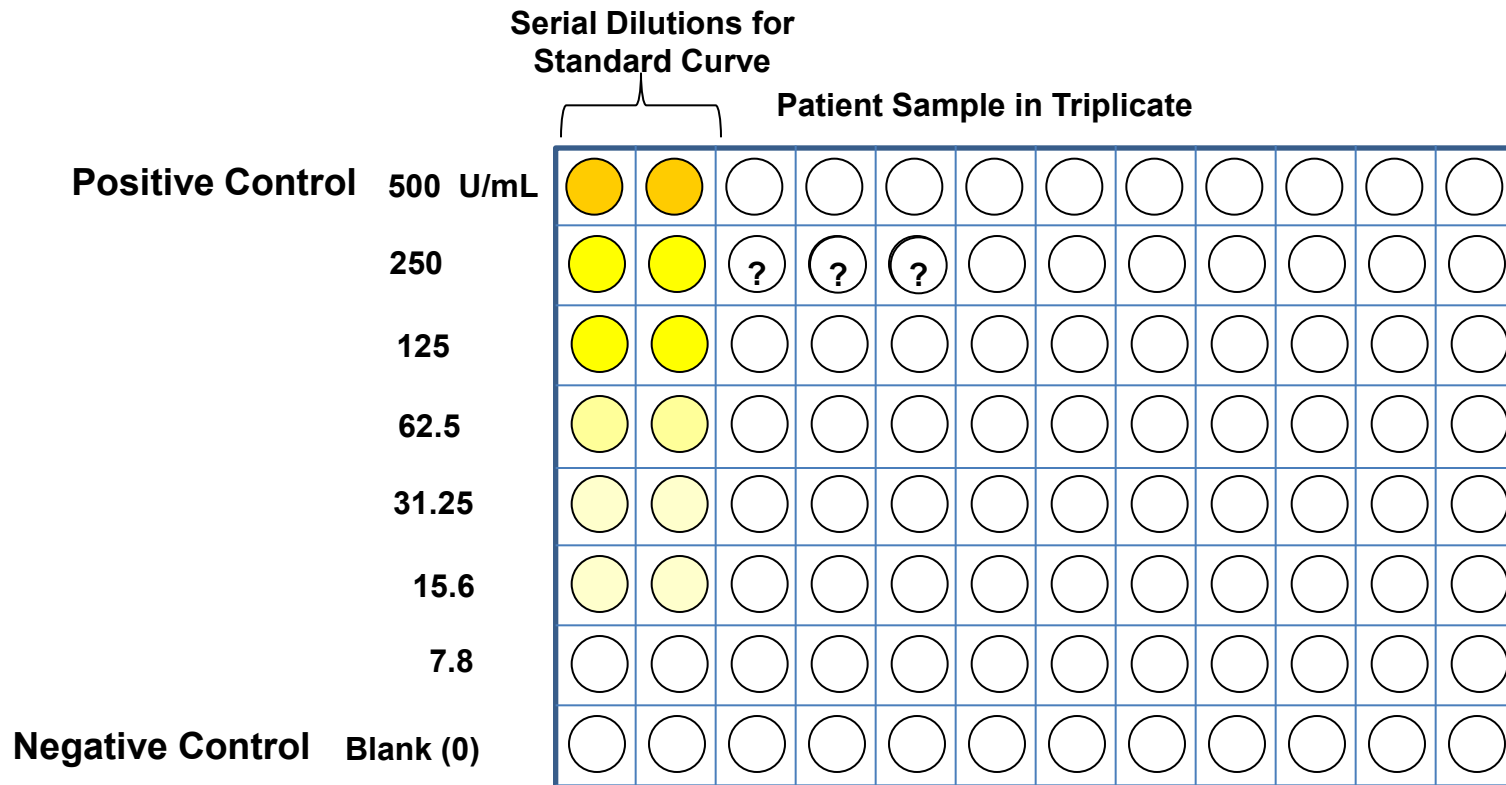
Highest Concentration



Lowest Concentration



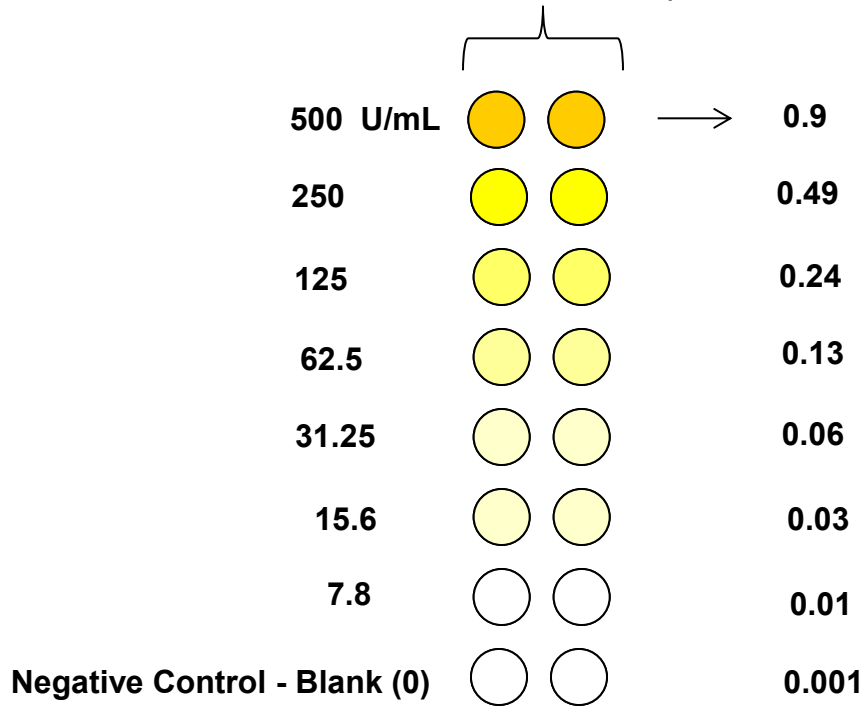
How do you figure out how much protein is in each patient sample?



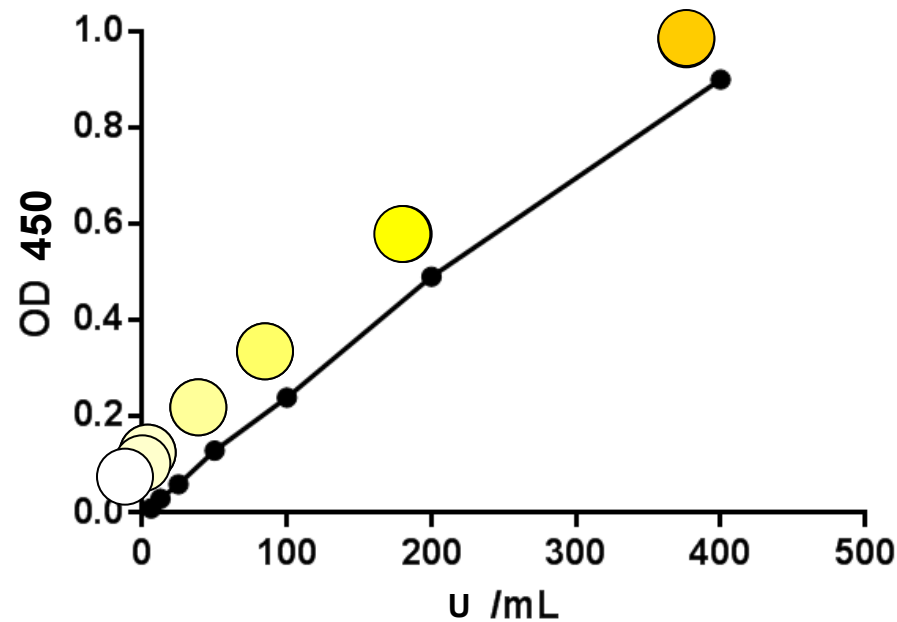
Convert Colors to Numerical Values

Measure the optical density of the samples on a Spectrophotometer using the appropriate wavelength of light

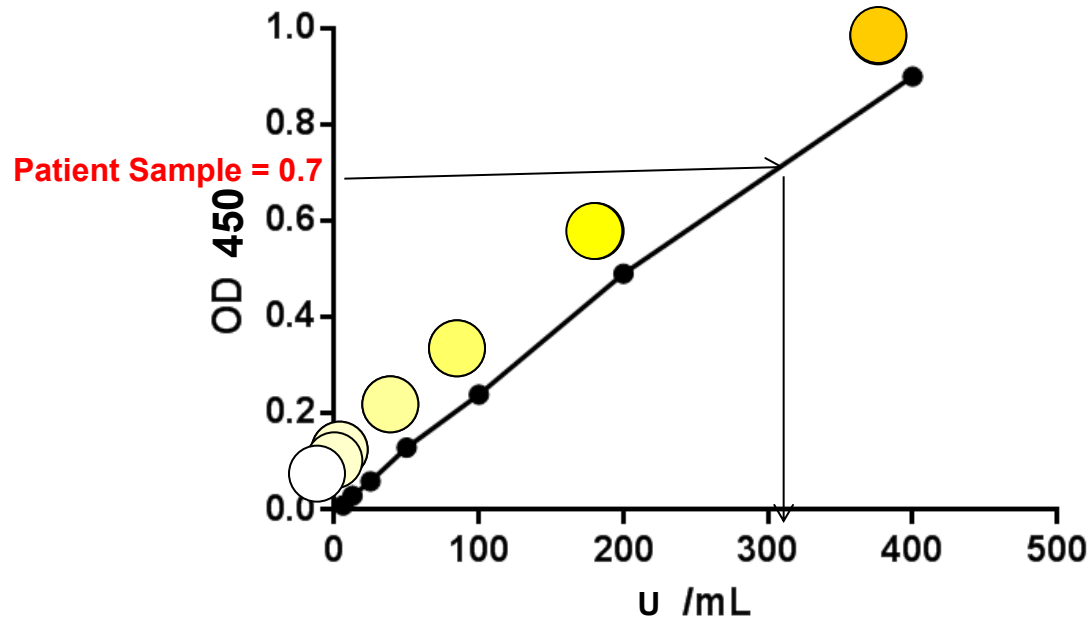
Standard Curve (Positive Control)



Graph the Standard Curve Values



Compare Patient Sample Values to the Standard Curve



Use the Equation of the line to determine the concentration of protein

$$y = mx + b$$

Where: y = OD value

m = the slope of the line

b = y intercept

x = $\mu\text{g/mL}$

Solve for X

$$y = 0.002x + 0.008$$

$$y = mx + b \longrightarrow mx = y - b \longrightarrow x = \frac{y - b}{m}$$

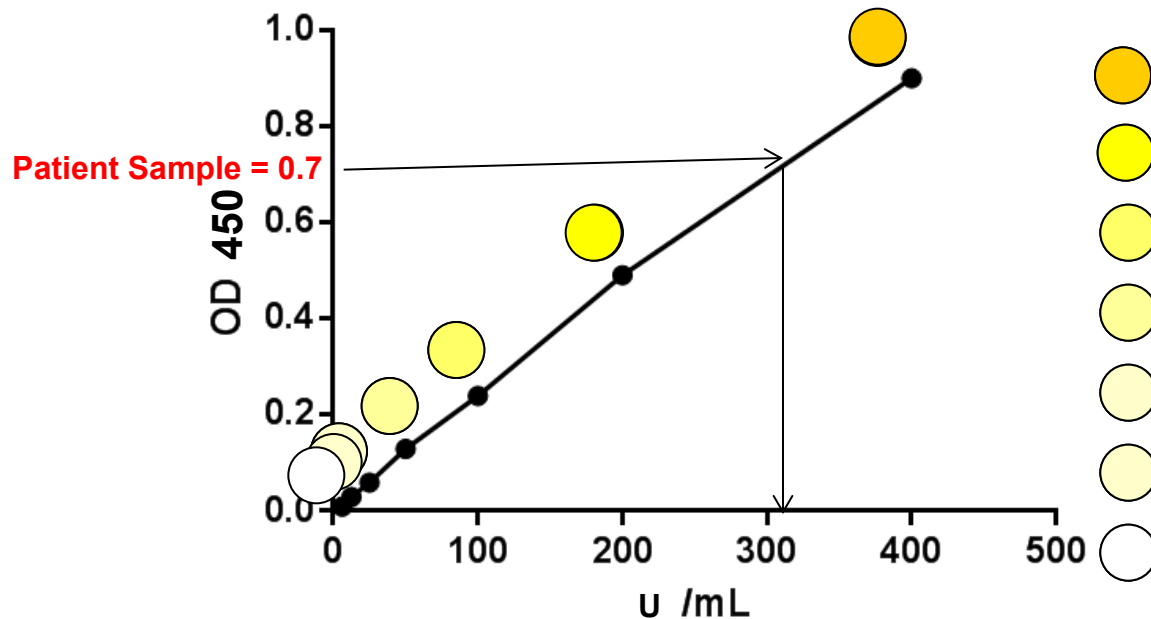
$$y = 0.7$$

$$m = 0.002$$

$$b = 0.008$$

$$x = \frac{0.7 - 0.008}{0.002} = 346 \text{ U/mL}$$

Standard Curve



Lesson

Sometimes you actually do use algebra
in your daily life!!!!!!!!!!!!!! 😊

Cancer Biomarker ELISA kits



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Cancer Biomarkers

Tumor markers are endogenous proteins or metabolites whose amounts or modifications are indicative of tumor state, progression characteristics, and response to therapies. They are present in tumor tissues or body fluids and encompass a wide variety of molecules, including transcription factors, cell surface receptors, and secreted proteins. Effective tumor markers are in great demand since they have the potential to reduce cancer mortality rates by facilitating diagnosis of cancers at early stages and by helping to individualize treatments. During the last decade, improved understanding of carcinogenesis and tumor progression has revealed a large number of potential tumor markers. It is predicted that even more will be discovered in the near future with the application of current technologies such as tissue microarrays, antibody arrays, and mass spectrometry. To apply these discoveries to patient care, vigorous validation and assay development for many tumor markers is currently underway. R&D Systems has a wide range of research reagents for use at every stage of the tumor marker development process. These products include a large collection of recombinant proteins, antibodies, and protein quantification kits (Quantikine ELISA kits and DuoSet ELISA development kits).

**Explore Our
CSC Marker
Diagram**

**Find the right
tools faster**

Cancer Biomarkers in Clinical Practice

[alpha-Fetoprotein/AFP](#)

[alpha-Methylacyl-CoA Racemase/AMACR](#)

[CA125/MUC16](#)

[ER alpha/NR3A1](#)

[ER beta/NR3A2](#)

[ErbB2/Her2](#)

[Kallikrein 3/PSA](#)

[Progesterone R/NR3C3](#)

[Progesterone R B/NR3C3](#)

[Thymidine Kinase 1](#)

Malaria ELISA kit



Malaria ELISA Kit

GenWay ID:	GWB-26MALA
Size:	1x96 Assays

Details for Malaria ELISA kit

Malaria ELISA The Malaria ELISA is intended for the qualitative determination of antibodies against Plasmodium in human serum or plasma (citrate).

Principal of the Assay The qualitative immunoenzymatic determination of antibodies against Plasmodium is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique.

Microtiter strip wells are pre-coated with Plasmodium antigens to bind corresponding antibodies of the specimen. After washing the wells to remove all unbound sample material, horseradish peroxidase (HRP) labeled anti-human IgG and IgM conjugate are added. This conjugate binds to the capture Plasmodium-specific antibodies. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate, which gives a blue reaction product. The intensity of this product is proportional to the amount of Plasmodium-specific antibodies in the specimen. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint color. Absorbance at 450 nm is read using an ELISA microwell plate reader.

Recombinant Antigen Recombinant CSP and MSP1 proteins from *Plasmodium vivax* and *Plasmodium falciparum*.

Lupus ELISA kit



J Clin Pathol. 1982 May; 35(5): 566–573.

PMCID: PMC497719

A simple, rapid ELISA method for the detection of DNA antibodies.

[R P Stokes](#), [A Cordwell](#), and [R A Thompson](#)

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This article has been [cited by](#) other articles in PMC.

Abstract

Employing an enzyme-linked immunosorbent assay (ELISA) technique the serum antibodies against native (double stranded) and denatured (single stranded) deoxyribonucleic acid (DNA) have been measured in various disease groups and a group of blood donor sera. The ELISA method has been compared with a radioimmunoassay method using native (double stranded) DNA as substrate antigen and a latex-fixation technique using particles coated with soluble deoxyribonucleoprotein (SNP). It is concluded that ELISA offers an economic and reliable alternative to isotope techniques for the assessment of antibody content in systemic lupus erythematosus (SLE) and related disease states for the clinical laboratory.

HIV ELISA kit

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Human anti-immunodeficiency virus antibody,HIV ELISA Kit

E. coli ELISA kit



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E.coli HCP ELISA kit

This kit was developed using broadly reactive polyclonal antibodies to the hundreds of different HCPs and is intended for use in determining the presence of E.coli host cell protein contamination in products manufactured by recombinant expression. Our assay validation has determined that the LOD for this kit is ~0.2ng/mL.

Catalog #	Size	Price	Qty.
F410	1 kit	\$650.00	<input type="text" value="1"/>

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