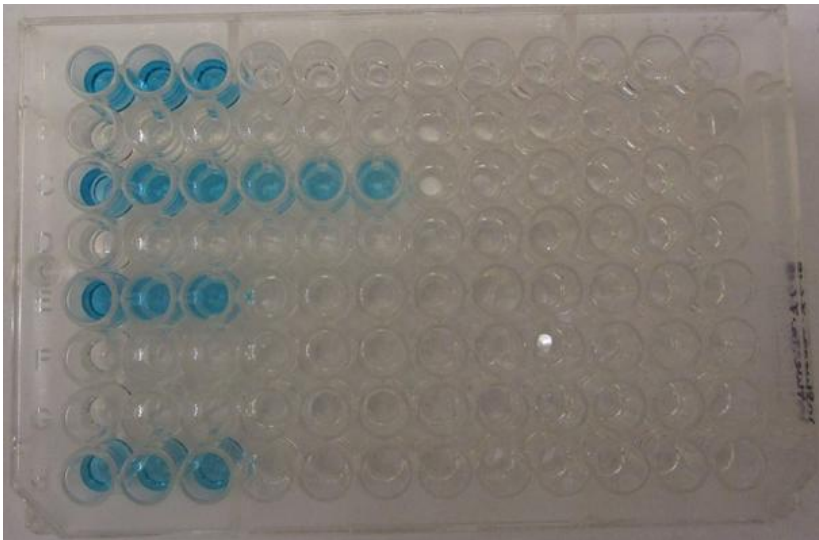


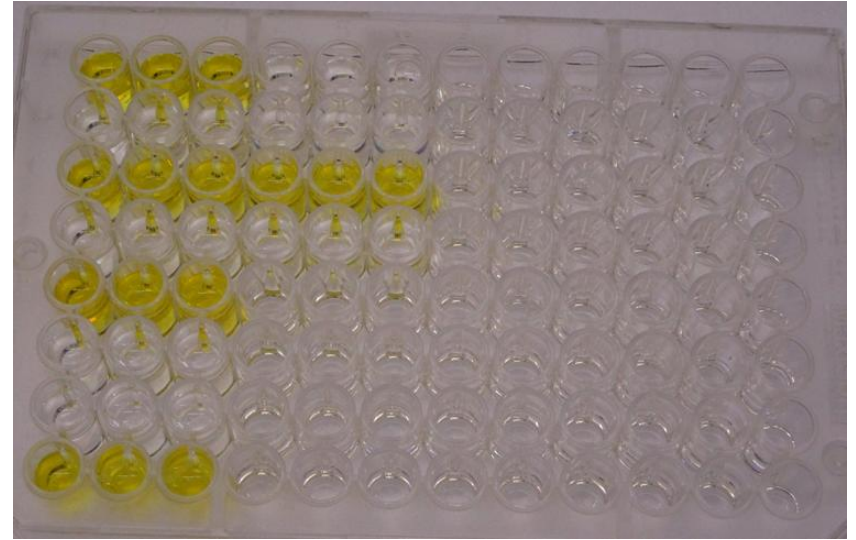
Enzyme-Linked Immunosorbent Assay (ELISA)

ELISA: Analyzing Many Samples at One Time

ELISA Plate During Development

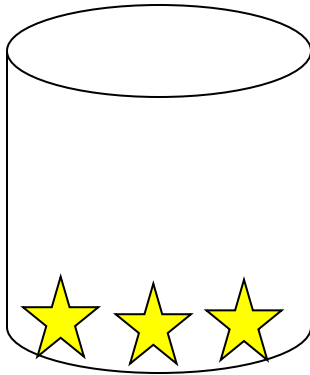


ELISA Plate After Development is Stopped

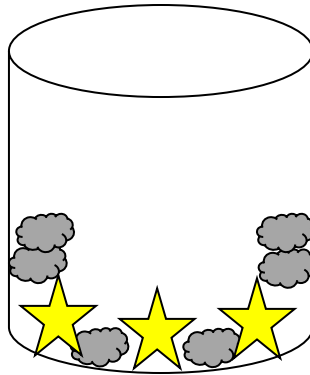


Direct ELISA

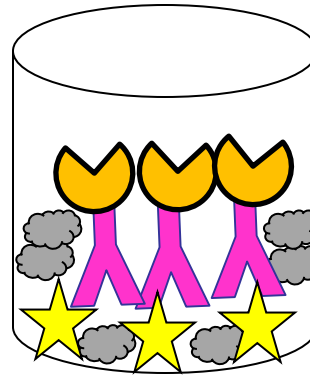
Antigen



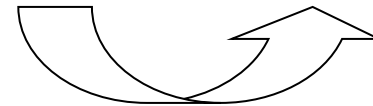
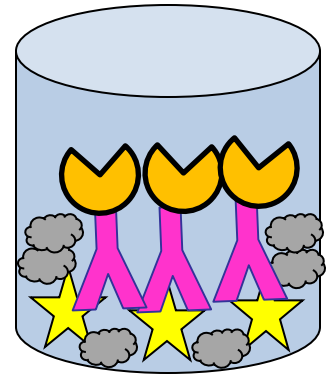
Block



HRP-Primary
Antibody



Color
Substrate



Wash

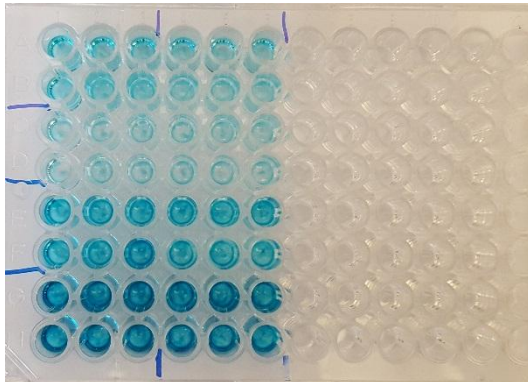
Horseradish Peroxidase (HRP)



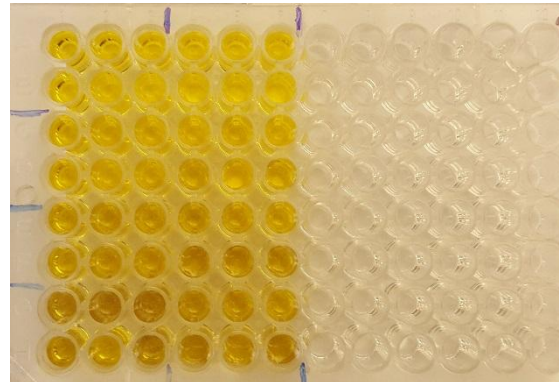
PDB ID 1W4W

- 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



Direct ELISA detects an analyte
(antigen/protein)

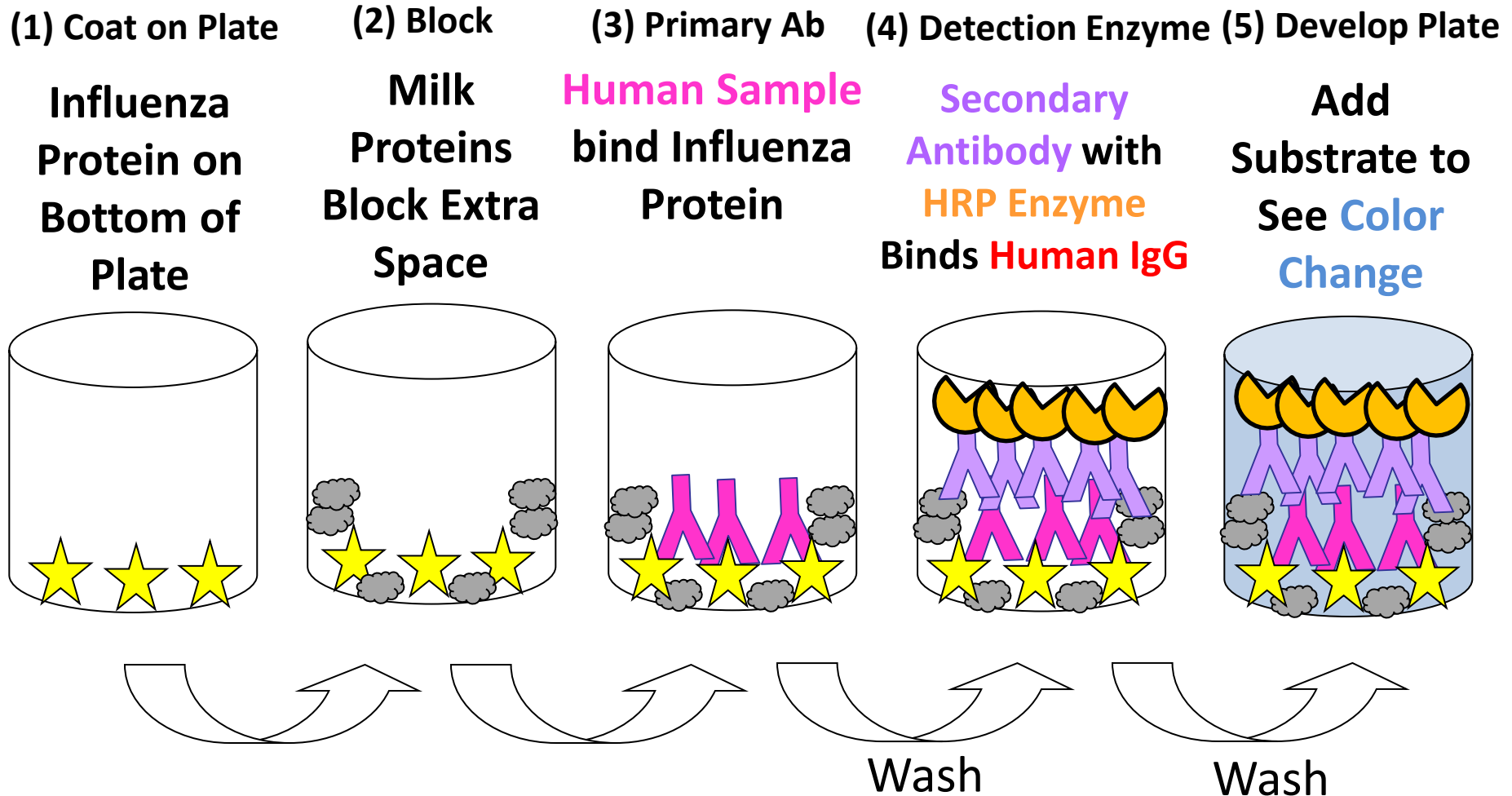
How would you design an ELISA to
measure *antibodies to a protein*?

Basic ELISA Steps:

ELISA Models

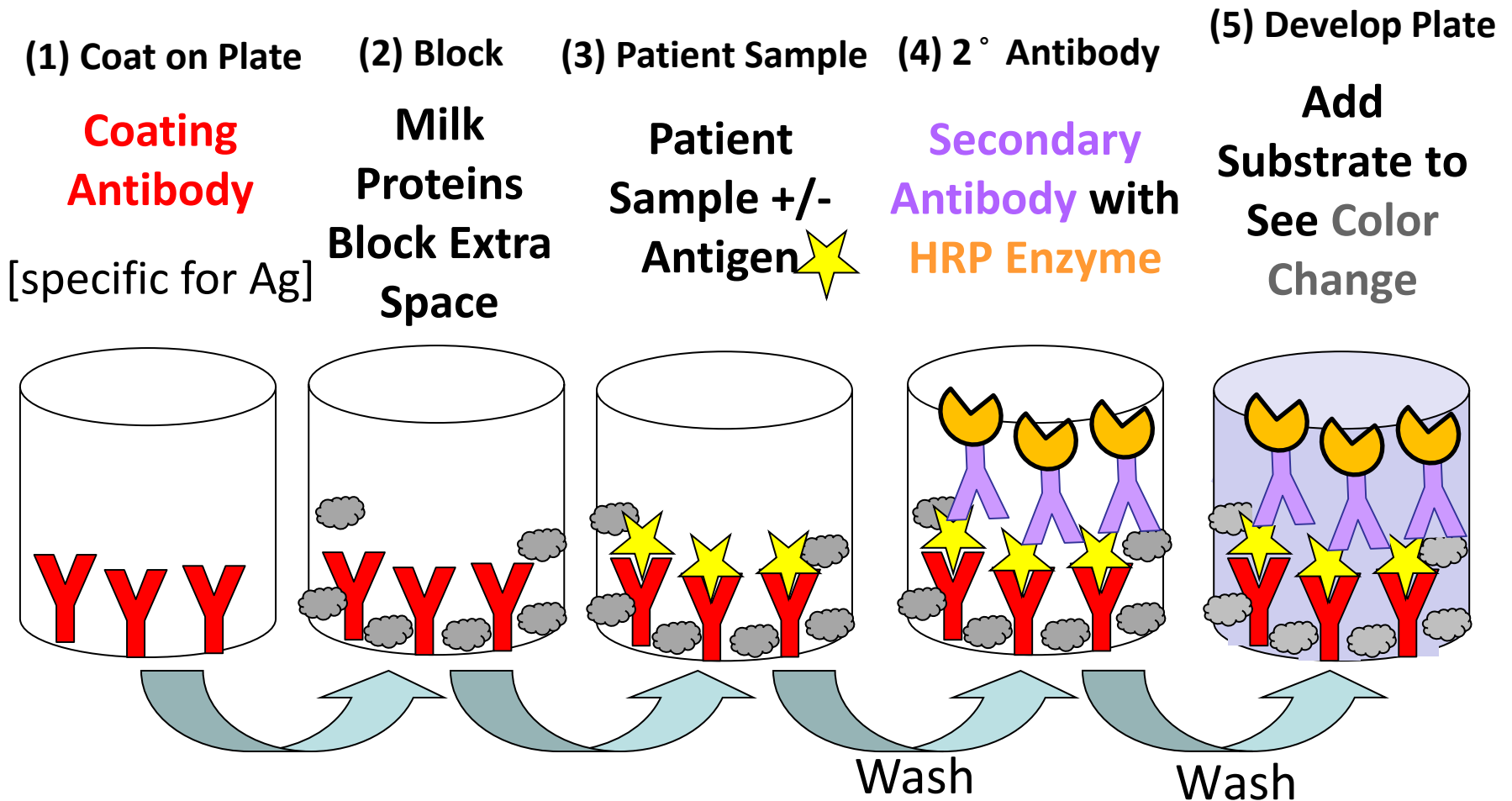
- **Coat** Plate with Antigen
- **Block** unbound space on plate to prevent non-specific binding
- Add patient **samples**, positive and negative controls
- **Wash** away unbound protein
- Add enzyme-conjugated **antibody**
- **Wash** away unbound enzyme-conjugated antibody
- **Develop** by adding substrate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

Measuring Influenza-Specific Antibodies by Indirect ELISA



How would you design an ELISA
to measure *a very small amount*
of protein?

Sandwich ELISA: Enzyme-Linked Secondary Antibody



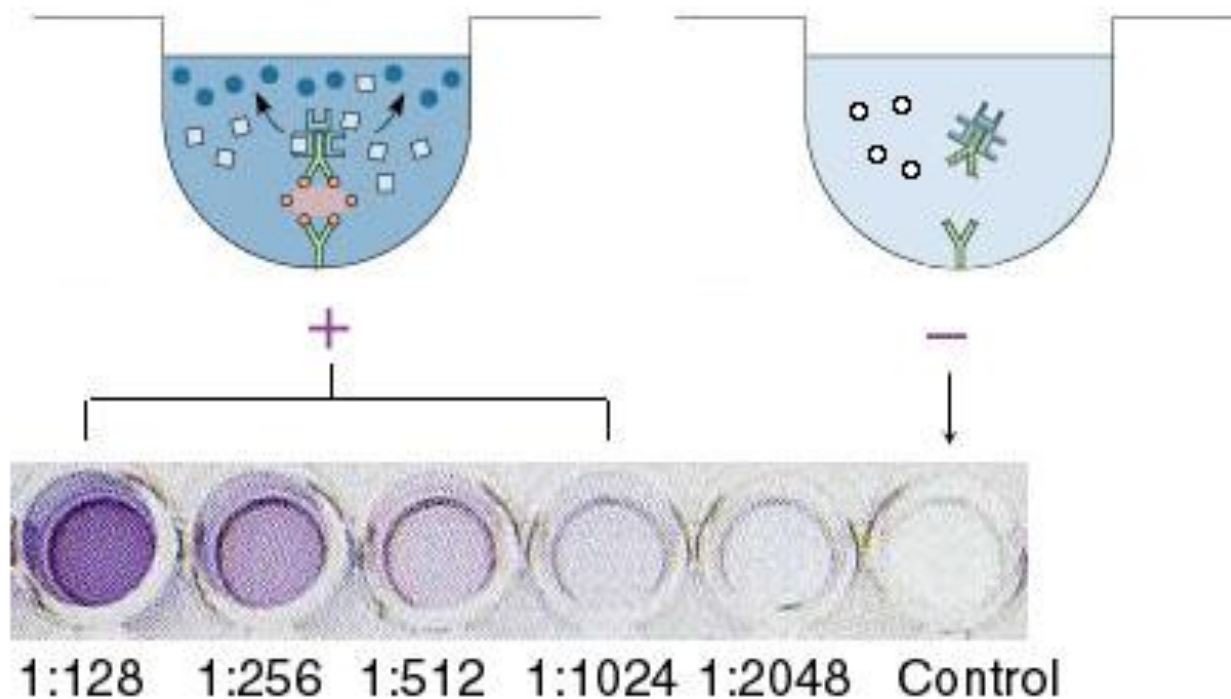
Review:

Types of ELISAs

- **Direct**—direct, single-step detection
- **Indirect**—indirect, two-step detection
- **Sandwich**—antigen “sandwiched” between 2 antibodies
- Various derivations and combinations have been and continue to be developed!

Use of Dilutions

- Dilutions can be used to calculate the ***unknown*** amount of a substance in a sample using samples of ***known*** concentrations























































































ELISA Plate Initial Read Out

Assume Positive Control Stock is 10 mg/ml = 10,000 µg/ml

Positive Controls
for Standard
Curve

Experimental Samples = ?

5,000 µg/ml	—————	1:2 dilution
2,500 µg/ml	—————	1:4 dilution
1,250 µg/ml	—————	1:8 dilution
625 µg/ml	—————	1:16 dilution
312 µg/ml	—————	1:32 dilution
156 µg/ml	—————	1:64 dilution
78 µg/ml	—————	1:128 dilution
39 µg/ml	—————	1:256 dilution

 Negative Controls

































































































ELISA Plate Read Out After Addition of Stop Solution

Positive Controls
for Standard
Curve

Assume Positive Control Stock
is 10 mg/ml = 10,000 µg/ml

Experimental Samples = ?

5,000 µg/ml	1:2 dilution
2,500 µg/ml	1:4 dilution
1,250 µg/ml	1:8 dilution
625 µg/ml	1:16 dilution
312 µg/ml	1:32 dilution
156 µg/ml	1:64 dilution
78 µg/ml	1:128 dilution
39 µg/ml	1:256 dilution

ELISA Plate Plan

Positive
Control Serial
Dilutions

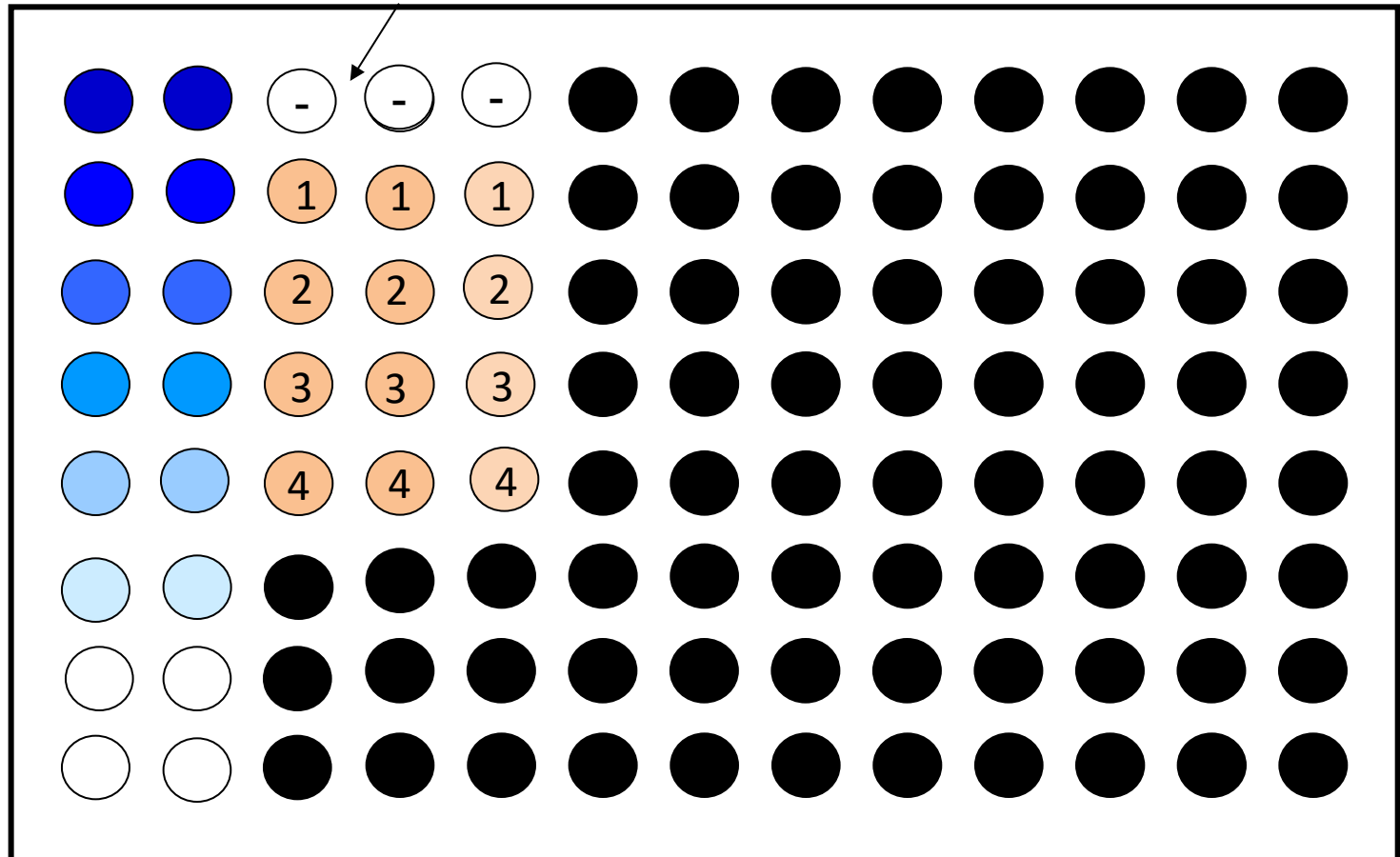
Negative
Controls

Experimental Samples

Highest
Concentration



Lowest
Concentration

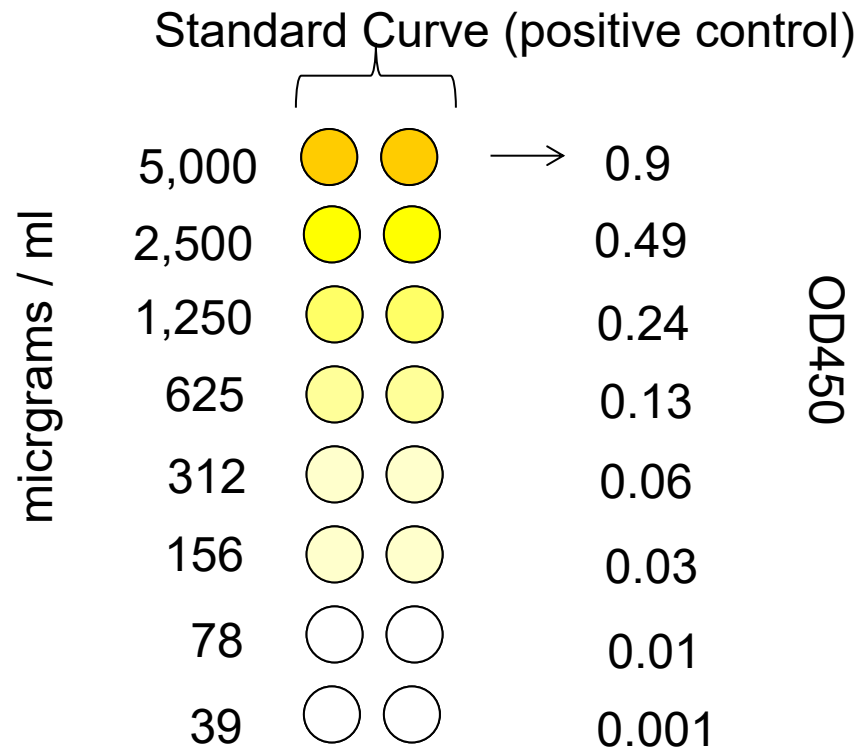


ELISA Plate Read Out

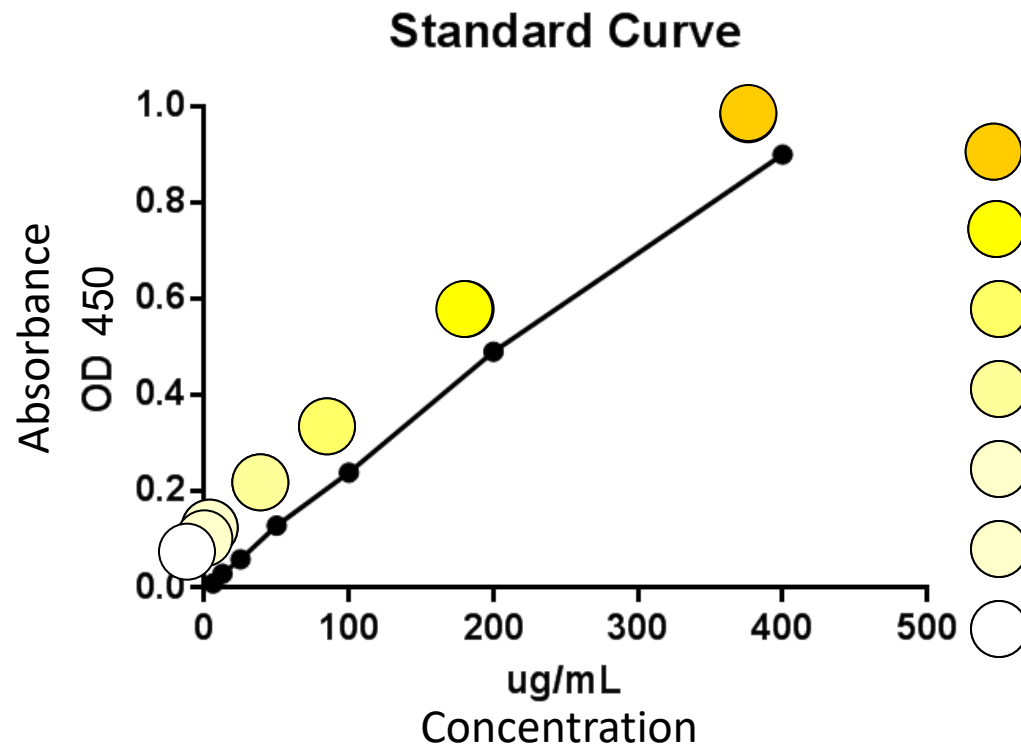
How do you figure out how much protein is
in each patient sample?

Convert Colors to Numerical Values

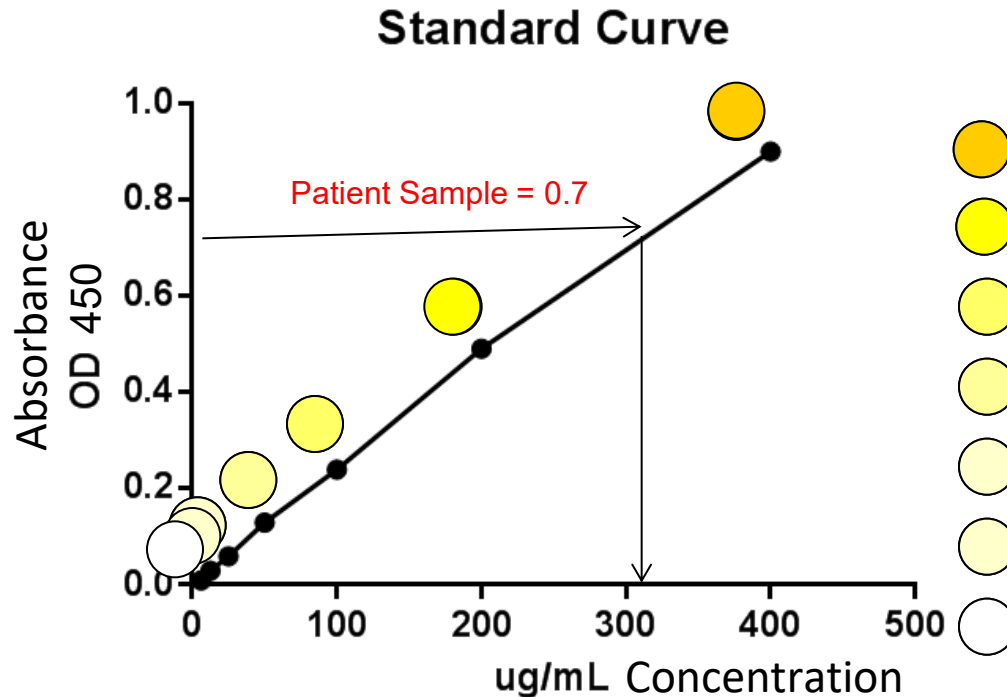
Measure the optical density of the samples on a Spectrophotometer using the appropriate wavelength of light (450 nm)



Graph the Standard Curve Values



Compare Patient Sample Values to the Standard Curve



What is the exact value?

Use the Equation of the line: $y = mx + b$

Where y = OD value

m = the slope of the line

b = y intercept

x = $\mu\text{g/mL}$ or relates back to the dilution of the positive control

Solve for X

$$y = 0.002x + 0.008$$

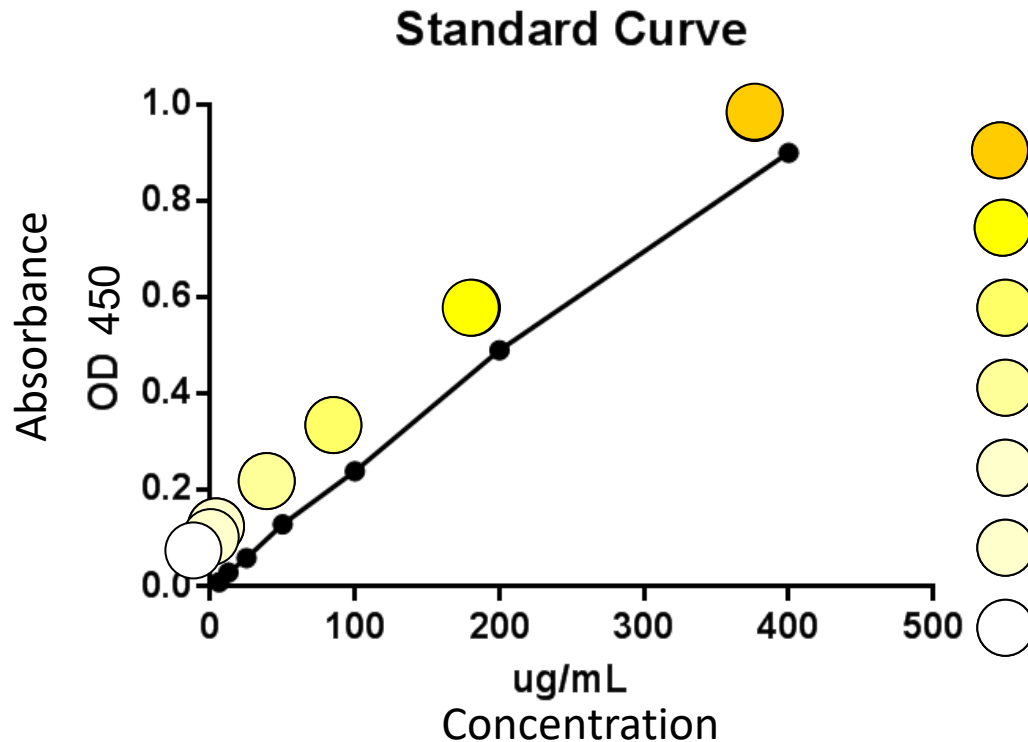
$$y = mx + b \longrightarrow mx = y - b \longrightarrow x = \frac{y - b}{m}$$

$$y = 0.7$$

$$m = 0.002$$

$$b = 0.008$$

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



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 re pre-coated with Plasmodium antigens to bind corresponding antibodies of the specimen. After washing the wells to remove all unbound sample material hoseradish 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 & *E. coli* ELISA Kits



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Human anti-immunodeficiency virus antibody,HIV ELISA Kit



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E.coli HCP 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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