# Absorbance Theory

# - Review -

***How to measure absorbance using a microplate reader and ELISA Basics***

# OD and Optics Theory

*Module 1*

## Goals

- Understand absorbance numbers

  1.572 2.853

  0.143 0.042

- Concepts
  - Absolute vs. Relative
  - Quantify vs. Qualify

- Understand why wavelength selection is important

- How BioTek verifies the reader is working correctly

- ELISA Basics

## First two concepts

- Absolute vs. Relative
  - Absolute = Absorbance
  - Relative = Fluorescence, Luminescence
- Quantify vs. Qualify
  - Quantify = Amount
  - Qualify = Good or Bad (or in between)

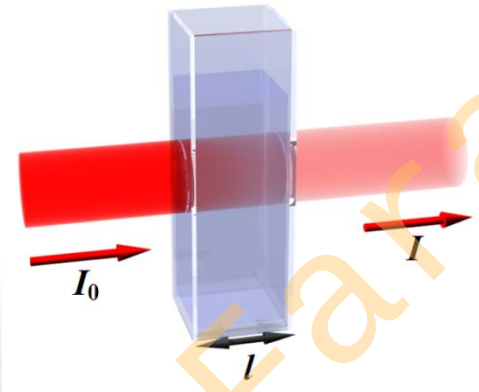
## What is Optical Absorption?

- Optical absorption is a process where **light energy** is **transformed** by a molecule into another form of energy, usually **heat**.

- To understand Absorbance, it's helpful to first understand transmittance.

## What is transmittance?






**Path length**

$$T = \frac{I}{I_0}$$

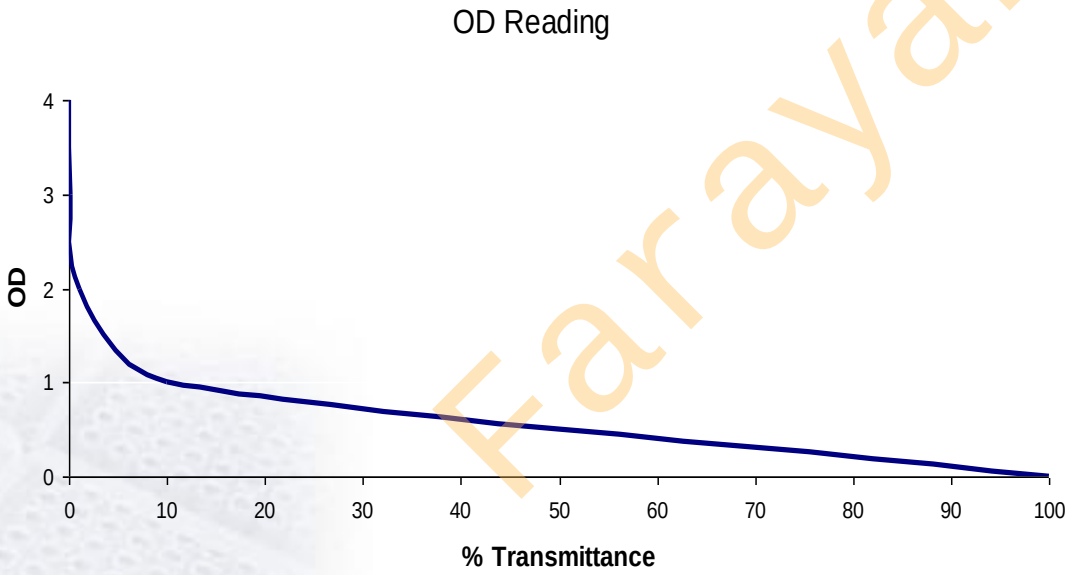
It's a Ratio

You need to measure both I and $I_o$ to calculate the %T

## What is Absorbance (AU)?



$$AU = -Log_{10}(\%T)$$



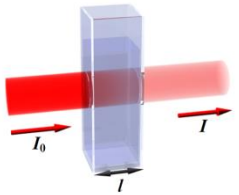

100% = 0.000 AU

10% = 1.000 AU

1% = 2.000 AU

0.1% = 3.000 AU

0.01% = 4.000 AU



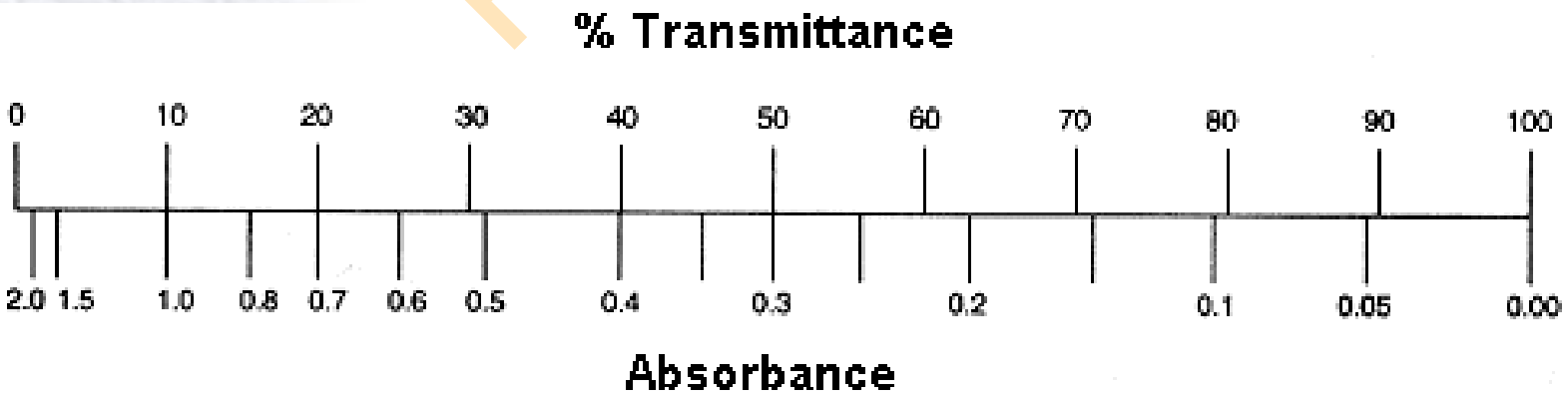

**Absorbance units (AU) are also called optical density (OD)**

# OD and Optics Theory

*Module 1*



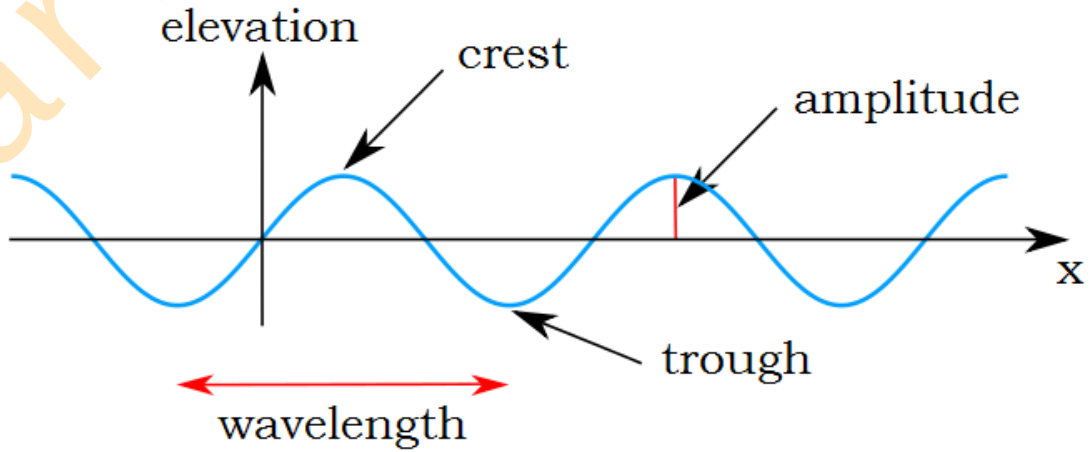
| % Transmittance | Absorbance |
|---|---|
| 100 | 0.000 |
| 90 | 0.046 |
| 80 | 0.097 |
| 70 | 0.155 |
| 60 | 0.222 |
| 50 | 0.301 |
| 40 | 0.398 |
| 30 | 0.523 |
| 20 | 0.699 |
| 10 | 1.000 |
| 5 | 1.301 |
| 2 | 1.699 |
| 1 | 2.000 |
| 0.5 | 2.301 |
| 0.1 | 3.000 |
| 0.01 | 4.000 |
| 0.001 | 5.000 |



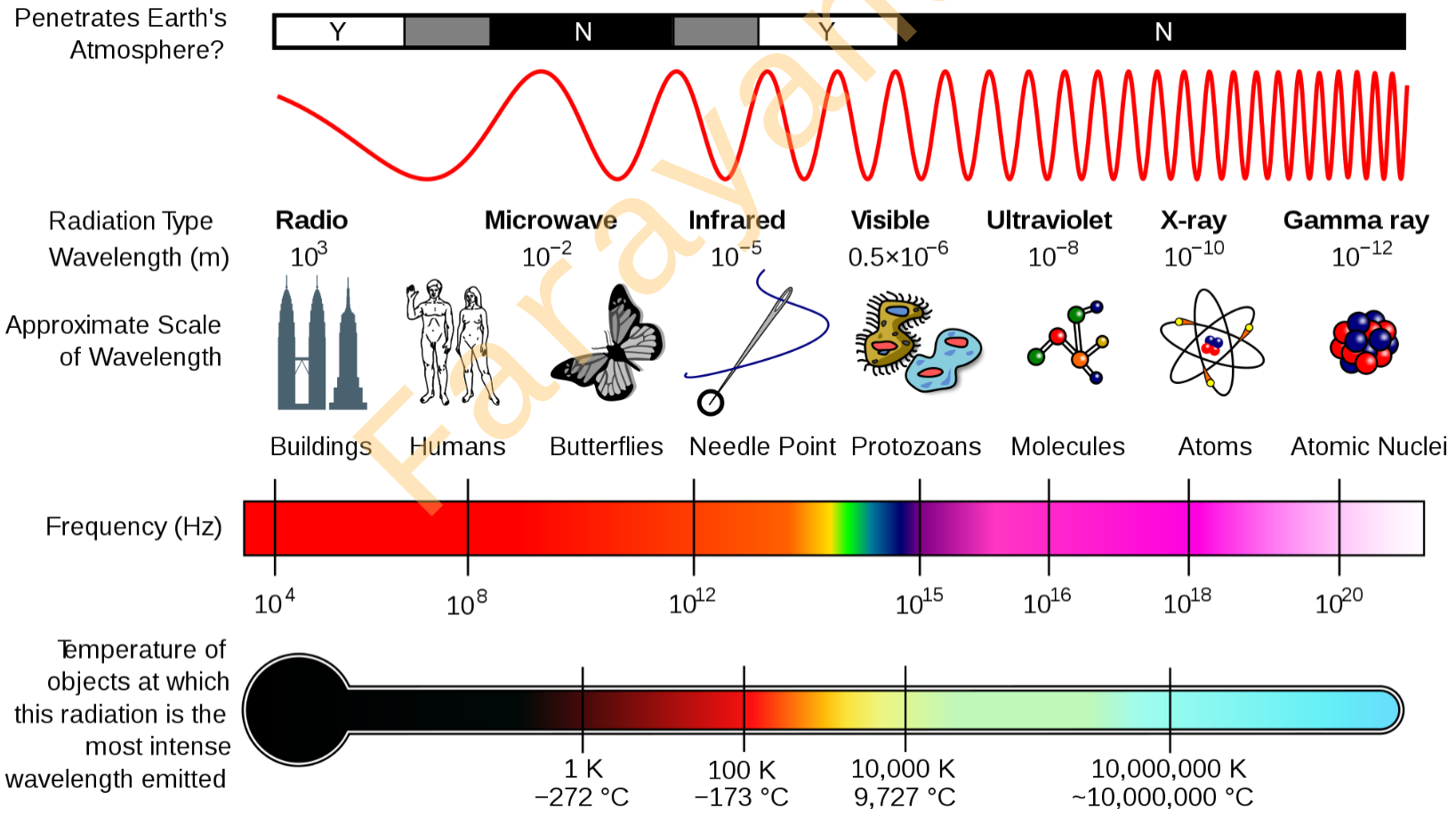
## Light as a wave

# Is color important?

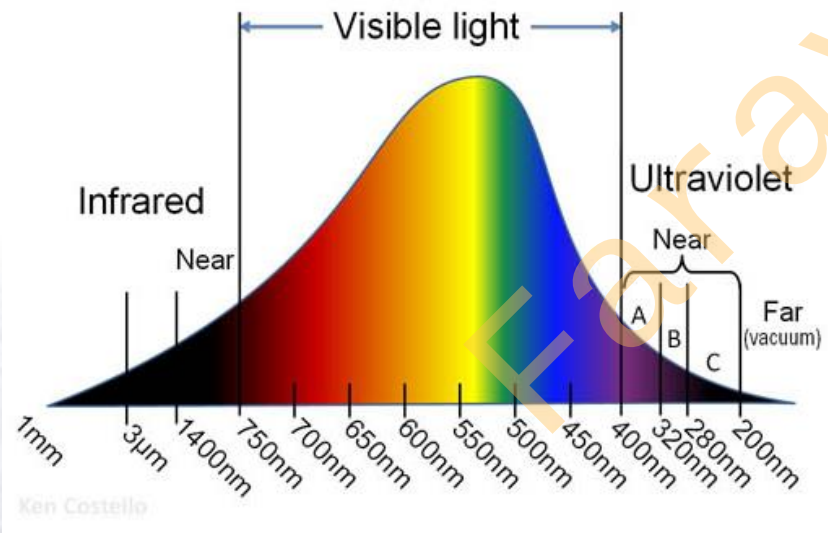
- **Wavelength**
- **Amplitude**



# Electromagnetic Radiation



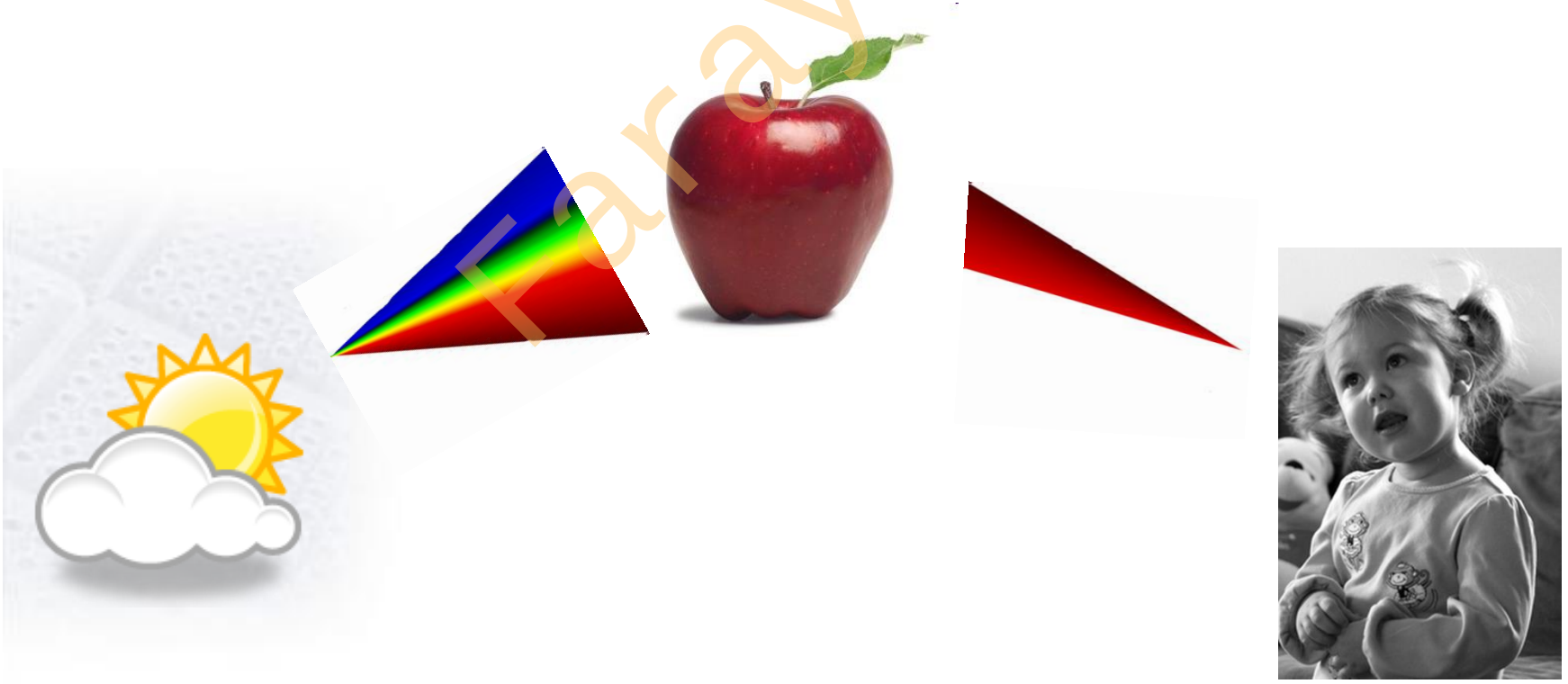
# Electromagnetic Radiation




| Color | Wavelength (nm) Approximate |
|---|---|
| Ultraviolet | 001-380 |
| Violet | 380-430 |
| Blue | 430-500 |
| Cyan | 500-520 |
| Green | 520-565 |
| Yellow | 565-590 |
| Orange | 590-625 |
| Red | 625-740 |
| Infrared | 750-1,000,000 |

## An object's color

• When white light falls on an object, the object **absorbs** certain waves and reflects others, this determines the object's color.



## Importance of color in the Assay

- **What color is the liquid in the microplate?**
- **What wavelength are you using to read the sample?**
- **Why?**

| Color | Wavelength (nm) Approximate |
|---|---|
| Ultraviolet | 001-380 |
| Violet | 380-430 |
| Blue | 430-500 |
| Cyan | 500-520 |
| Green | 520-565 |
| Yellow | 565-590 |
| Orange | 590-625 |
| Red | 625-740 |
| Infrared | 750-1,000,000 |

## Components of the absorbance instrument

| | | |
|---|---|---|
| 1. | Light source | Tungsten Halogen |
| 2. | Wavelength selection device | Band Pass Filters |
| 3. | Detector | Photodiode |
| 4. | Method to acquire $I_o$ | Computer |

## Qualification

- Absorbance Test Plate
  - Solid state neutral density glass
- Liquid Tests
  - Color solution

## Qualification

### Absorbance Test Plate

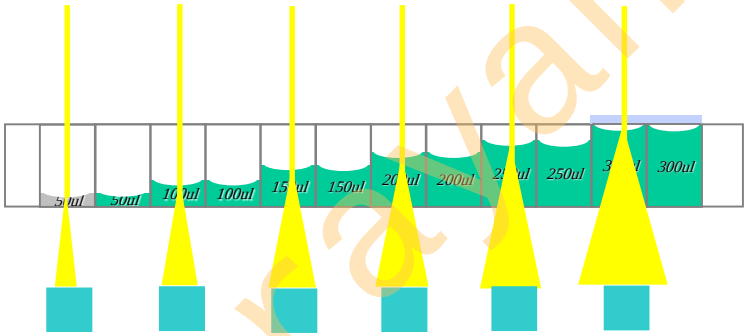
- Alignment
- Accuracy
- Repeatability
- Turnaround
- Linearity
- Wavelength Accuracy (Not use with the ELx808)

## Qualification

### Liquid Tests

- Liquid testing differs from testing with the Absorbance Test Plate because liquid in the wells has a **meniscus**.
- The optics characteristics may differ in these two methods.

## Liquid Tests






**The curved meniscus acts like a lens.**

Microplate (Flat Bottom)

Fluid used is DI water from 50ul to 300ul

**To reduce this problem, we recommend shaking the plate for 5 minutes or letting it sit 20 minutes before reading the plate.**

## Liquid Tests

- Liquid Test 1
  - Alignment, Accuracy, Repeatability, and channel-to-channel variability
- Liquid Test 2 (Not needed if using Absorbance Test Plate)
  - Linearity, Repeatability, and Alignment
  - Uses a series of solutions of varying absorbencies
- Liquid Test 3
  - An optional test offered for sites that must have proof of linearity at wavelengths lower than those attainable with the Absorbance Test Plate
  - This test is considered optional because the reader has good “front end” linearity throughout its wavelength range

# Liquid Test 1

## Absorbance Liquid Test 1

**Mean ODs for five reads:**

*"Normal"*

| | 1 | 2 |
|---|---|---|
| A | 1.988 | 1.023 |
| B | 2.008 | 1.023 |
| C | 2.006 | 1.031 |
| D | 2.003 | 1.024 |
| E | 2.010 | 1.034 |
| F | 2.016 | 1.032 |
| G | 2.016 | 1.029 |
| H | 2.009 | 1.030 |

*"Turnaround"*

| | 11 | 12 |
|---|---|---|
| A | 1.029 | 2.004 |
| B | 1.027 | 2.021 |
| C | 1.029 | 2.011 |
| D | 1.034 | 2.020 |
| E | 1.025 | 2.010 |
| F | 1.034 | 2.022 |
| G | 1.022 | 2.010 |
| H | 1.019 | 1.999 |

**Results:**

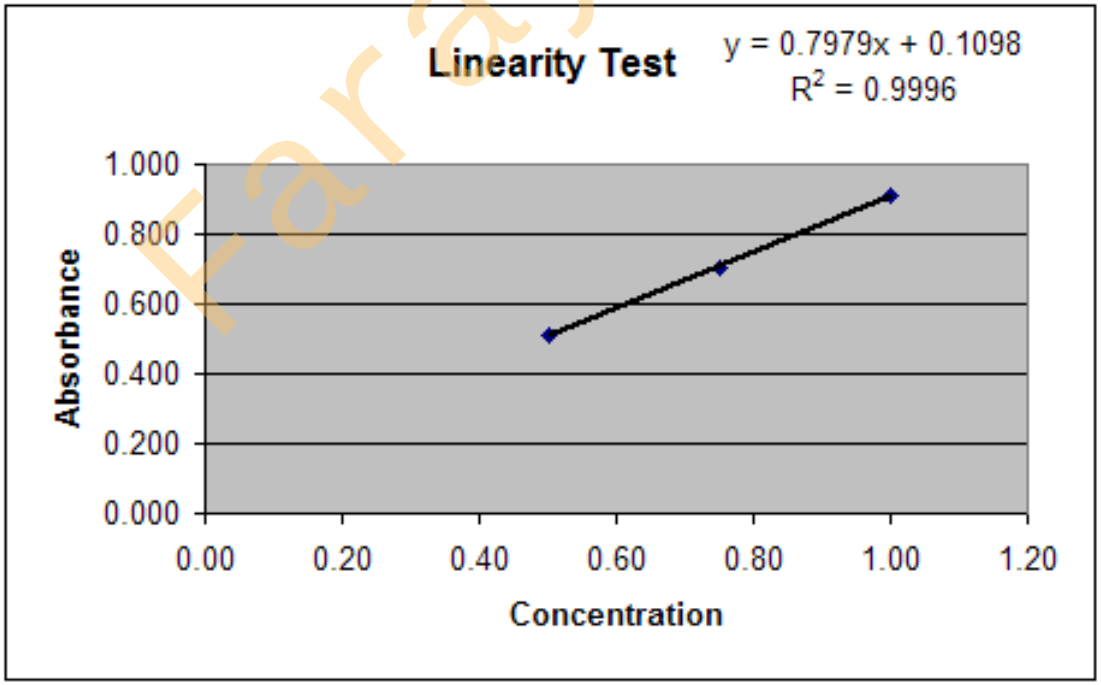
| Wells for Comparison | Spec | Range Low | Range High | Result |
|---|---|---|---|---|
| A1/H12 | +/- 0.030 | 1.9581 | 2.0179 | PASS |
| B1/G12 | +/- 0.030 | 1.9779 | 2.0381 | PASS |
| C1/F12 | +/- 0.030 | 1.9759 | 2.0361 | PASS |
| D1/E12 | +/- 0.030 | 1.9730 | 2.0330 | PASS |
| E1/D12 | +/- 0.030 | 1.9799 | 2.0401 | PASS |
| F1/C12 | +/- 0.030 | 1.9858 | 2.0462 | PASS |
| G1/B12 | +/- 0.030 | 1.9858 | 2.0462 | PASS |
| H1/A12 | +/- 0.030 | 1.9789 | 2.0391 | PASS |
| A2/H11 | +/- 0.020 | 1.0028 | 1.0432 | PASS |
| B2/G11 | +/- 0.020 | 1.0028 | 1.0432 | PASS |
| C2/F11 | +/- 0.020 | 1.0107 | 1.0513 | PASS |
| D2/E11 | +/- 0.020 | 1.0038 | 1.0442 | PASS |
| E2/D11 | +/- 0.020 | 1.0137 | 1.0543 | PASS |
| F2/C11 | +/- 0.020 | 1.0117 | 1.0523 | PASS |
| G2/B11 | +/- 0.020 | 1.0087 | 1.0493 | PASS |
| H2/A11 | +/- 0.020 | 1.0097 | 1.0503 | PASS |

*Accuracy specification:*
*± 1.0% ± 0.010 Abs from 0 to 2.000 Abs*

# Liquid Test 3

**Average Mean for each dye concentration ('Y'):**

| | X | Y |
|---|---|---|
| Wells A5-H6 Low-level (50%): | 0.50 | 0.511 |
| Wells A3-H4 Mid-level (75%): | 0.75 | 0.704 |
| Wells A1-H2 High-level (100%): | 1.00 | 0.910 |




*** Also checks Standard Deviation**

## ELISA

- **Definitions**
- **Enzyme-Linked Immunosorbent Assay**
- **ELISA Applications**
- **Sumanas ELISA Video**

# ELISA Definitions

**Antibody**
Antibodies are a type of protein. They are produced by the immune system in response to foreign substances that may be a threat to the body -- such as chemicals, virus particles, spores, or bacterial toxins. These foreign substances are called antigens.
Each type of antibody is unique and defends the body against one specific type of antigen.

**Antigen**
An antigen is any substance that causes your immune system to produce antibodies against it. The antigen may be a foreign substance from the environment (such as chemicals, bacteria, viruses, or pollen) or formed within the body (such as bacterial toxins or tissue cells).

**Enzyme**
Enzymes are complex proteins that cause a specific chemical change in other substances without being changed themselves. Enzymes can be found in every organ of the body. For example, they can change starches, proteins, and sugars into substances the body can digest. Blood clotting is another example of enzymes at work.
Enzymes exist in the mouth (saliva), stomach (gastric juice), and intestines (pancreatic juice, intestinal juice, and intestinal mucosa).

## ELISA

- **Enzyme-Linked Immunosorbent Assay**
  - Alternate Names : EIA, Enzyme-Linked Immunoassay
- This versatile test is widely used in the clinical laboratory
- It allows your health care provider to:
  - test your blood with an antigen (e.g., virus or bacteria) to see if your immune system recognizes it as something it has seen before, or
  - test your blood with an antibody to see if a particular substance like a hormone (an antigen) is present in your system.

# ELISA

## ELISA Applications

- HIV
- Lyme disease
- Cell death detection
- Cell Proliferation
- Blood Donor Screening
- Syphilis
- Hepatitis
- Bird Flu
- H1N1
- hCG Pregnancy Test (Human Chorionic Gonadotropin)
- Anabolic Steroids
- Narcotics
- LSD
- Drug Facilitated Sexual Assault

The list goes on and on….

## ELISA

**Sumanas ELISA Video**

## Questions

Questions?

Thank You