Colorimetric Assays:
A Scientific Method for Determining the Concentration of Vitamin C

This is a **PREVIEW** of Colorimetric Assays: A Scientific Method for Determining the Concentration of Vitamin C, Field Trip Version (3 Lessons).

The full curriculum includes two classroom lessons and one lesson in the Science Discovery Lab in downtown Seattle where students perform of a colorimetric assay experiment.

Teachers requesting the field trip version must be located in Western Washington.

To request the full version of this curriculum at no cost, go to
https://www.adventurelab.org/nih-sepa-grant-project.aspx
and fill out the request form.

https://freesvg.org/chemical-molecule-3d-graphics

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How to decide which version is best for your classroom:

Where are you located?	How much time is available?	How many classes are participating?	Version	Description
Western Washington	3 lessons, including a 1-hour mobile lab visit	3-5 (30 students max per class)	Science Adventure Lab (Mobile Lab) Version	3 days, includes a mobile Science Adventure Lab visit to your school
Puget Sound area	3 lessons, including a 2 ½-hour field trip	1 (32 students max)	Science Discovery Lab (Field Trip) Version	3 days, includes a field trip to the Science Discovery Lab in downtown Seattle; transportation assistance is available
Any location	2 lessons	Not limited	Classroom Version (coming soon)	Classroom activities (2 days)

Updated May 2023

Colorimetric Assays: A Scientific Method for Determining the Concentration of Vitamin C

General Description

This is a three-lesson unit, with two of the lessons taking place in the classroom and one at the Science Discovery Lab located at Seattle Children's Research Institute in downtown Seattle.

Students will learn how scientists use assays to detect and quantify substances. Specifically, they will learn about a type of assay called a colorimetric assay where the results are seen as color change that occurs due to a chemical reaction. To interpret the colorimetric assay, students will learn how to create a color scale.

To prepare for performing the assay at the Science Discovery Lab, the teacher will lead students in an activity that demonstrates the principle of creating standards by making a series of dilutions that follow a consistent mathematical pattern.

While in the lab, students will create a stock solution, controls, and standards by diluting the stock solution. Students will test all solutions, including two unknown solutions, using a test strip containing a reagent that detects Vitamin C. Students will observe and record the color of the test strips and utilize that data in the classroom for the final lesson.

Using the data from the experiment, students will create a color scale that represents the relationship between color and concentration and use the scale to determine the concentration of Vitamin C in the unknowns. The lesson concludes with answering the essential question "How can a colorimetric assay be used to determine the concentration of a substance in an unknown solution?" and completing a Claim, Evidence, Reasoning framework. This curriculum unit is designed for students in Grade 8.

The Science Adventure Lab

The Science Adventure Lab is a custom-built, 45-foot, wheelchair-accessible, mobile laboratory equipped with research-grade equipment and space for up to 30 students. The mobile lab travels to schools across Washington state providing innovative, hands-on science curriculum to students in grades 4-8.

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The Science Discovery Lab

Lesson 4 takes place in the Science Discovery Lab and is taught by scientists from Seattle Children's Research Institute. The Science Discovery Lab is a next-generation science classroom where students can do innovative curriculum in a state-of-the-art laboratory embedded in the research institute. Field trips are three hours in duration.

Please visit www.seattlechildrens.org/scienceeducation for more information.

Overview and Standards

Performance Expectation	Disciplinary Core Ideas	Science and Engineering Practices	Crosscutting Concepts	Vocabulary
MS-PS1-2: Analyze and interpret data on the properties of substances before and after the substances interact to determine if a chemical reaction has occurred.	**PS1.B: Chemical reactions** Substances react chemically in characteristic ways. (MS-PS1-2)	**Analyzing and Interpreting Data** Analyze and interpret data to determine similarities and differences in findings. (MS-PS1-2)	**Patterns** Patterns in rates of change and other numerical relationships can provide information about natural and human designed systems. Graphs, charts, and images can be used to identify patterns in data.	Assay Color scale Colorimetric assay Concentration Control Dilution Reagent Standards Stock solution Vitamin C

Nature of Science

Understandings about the Nature of Science: Themes closely associated with Scientific and Engineering Practices	
Categories	**Middle School**
Scientific Investigations Use a Variety of Methods	Science investigations use a variety of methods and tools to make measurements and observations. Science investigations are guided by a set of values to ensure accuracy of measurements, observations, and objectivity of findings.
Scientific Knowledge is Based on Empirical Evidence	Science knowledge is based upon logical and conceptual connections between evidence and explanations. (MS-PS1-2).

Understandings about the Nature of Science: Themes closely associated with Crosscutting Concepts	
Categories	**Middle School**
Science is a Way of Knowing	Science is a way of knowing used by many people, not just scientists.
Scientific Knowledge Assumes an Order and Consistency in Natural Systems	Science assumes that objects and events in natural systems occur in consistent patterns that are understandable through measurement and observation.

Source: Appendix H – Understanding the Scientific Enterprise: The Nature of Science in the Next Generation Science Standards www.nextgenscience.org/resources/ngss-appendices.

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Lesson 1
Introduction to Assays

Lesson 1 Overview

The lesson starts with students watching a video about how scientists use assays to detect and quantify substances and the different types of assays. Students learn about a specific type of assay called a colorimetric assay where the results are seen as a color change that occurs due to a chemical reaction. The teacher will lead students in an activity that demonstrates the principle of creating standards by making a series of dilutions that follow a consistent mathematical pattern. Finally, students will watch a short video about laboratory safety and about the essential equipment they will use while performing the assay in the Science Discovery Lab. *This lesson should take between 50-55 minutes of classroom time.*

Guiding Question

What is an assay and how is it used to detect and quantify substances?

Outcomes and Objectives

Specific Learning Outcomes
Students will be able to:

1. Define the vocabulary term assay and describe how they are used to detect and quantify substances.
2. Describe how a colorimetric assay produces a color change.
3. Identify the mathematical pattern for a series of dilutions.

Key Vocabulary

- **Assay**
- **Color scale**
- **Colorimetric assay**
- **Concentration**
- **Dilution**
- **Reagent**
- **Standards**
- **Stock Solution**
- **Vitamin C**

Essential Laboratory Equipment

Mortar and Pestle	Weigh Boat	Vortex mixer
Analytical Scale	Graduated Cylinder	Micropipette
Scoopula		

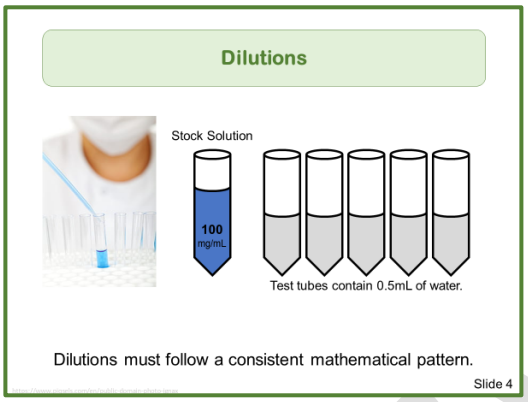
Materials Needed

- If students have an existing science lab notebook or composition book, it is recommended they use that to show their work. Loose paper could be used as an alternative.
- PowerPoint presentation with videos for Lesson 1.
- Teacher manual.
- Dilution Practice Activity.
- Pencils.

SAMPLE

Slide 4 — Lesson 1 — *3 minutes*

Slide 4



How to Dilute a Stock Solution to Make Standards.

4A. *Say This:*

Let's talk more about how to make stock solutions and standards that you can use in an assay.

Here's an example: You are working in a lab, and you need to make a stock solution that contains 100mg of sugar in each milliliter of water, which is a concentration of 100mg/mL. Your job is to use this stock solution to make five solutions with different concentrations that will serve as your ***standards.*** *In a colorimetric assay, these standards will help create the color scale that is necessary to show the relationship between color and concentration. How would one make the dilutions to create the standards? Let me explain.*

4B. Describe to students the best way to dilute a solution in order to make standards is to follow a consistent mathematical pattern. This allows you to know exactly what the concentration is in each tube.

Background: Click to the next slide to show students an example.

Lesson 2: Science Discovery Lab
Performing a Colorimetric Assay for Vitamin C

Lesson Overview
In this laboratory activity, students will use authentic scientific equipment to perform a colorimetric assay for Vitamin C. They will create a stock solution and use that to make a series of dilutions that will serve as standards. Students will collect data needed to create a color scale by testing each standard with a test strip that changes color due to a chemical reaction between a reagent on the strip and Vitamin C in the solutions. While performing the assay, students will learn how to use scientific tools including a mortar and pestle, analytical scale, micropipette, and vortex mixer. *This lesson is part of the 2.5-hour field trip that takes place in downtown Seattle in the Science Discovery Lab. The lesson is taught by scientists from Seattle Children's Research Institute.*

Guiding Question
How can a colorimetric assay be used to determine the concentration of a Vitamin C?

Outcomes and Objectives
Specific Learning Outcomes
Students will be able to:

1. Use the scientific tools available in the Science Discovery Lab to create a stock solution of Vitamin C, five standards, and two controls.
2. Make a series of dilutions that follow a repeatable mathematical pattern to make five standards with known concentrations.
3. Perform a colorimetric assay using a test strip that detects Vitamin C.

Key Vocabulary
- **Colorimetric assay**
- **Concentration**
- **Control**
- **Dilution**
- **Reagent**
- **Standards**
- **Stock Solution**

Protocol for Science Adventure Lab Team (Information for Teachers)
During the lesson on the Science Adventure Lab, the Seattle Children's instructors will complete the following:

1. Review objective of the assay.
2. Review use of scientific tools.
3. Lead students through practice activity with the pipette.
4. Instruct students to complete the assay.
5. Students will test two unknown solutions.
6. An image of the results will be given to the teacher for use in the next lesson.

Lesson 3
Interpreting the Results of the Assay

Lesson Overview
In this lesson, students will calculate the concentration of Vitamin C in the stock solution and each standard. Using the standards, students will build the color scale that represents the relationship between the concentration of Vitamin C and color. They will use the color scale to determine the concentration of Vitamin C in the unknown solutions. Finally, they will state a final claim, compile all the evidence for the claim, and provide their reasoning about how a colorimetric assay can be used to determine the concentration of a substance in an unknown solution. *This lesson should take between 55-60 minutes of classroom time.*

Guiding Question
How is the color scale used to determine the concentration of Vitamin C in unknown solutions?

Outcomes and Objectives
Specific Learning Outcomes
Students will be able to:

1. Calculate the concentration of Vitamin C in the stock solution and each standard.
2. Create a color scale that represents the relationship between concentration of Vitamin C in each standard and the color of the test strip.
3. Use the color scale to determine the concentration of Vitamin C in unknown solutions.

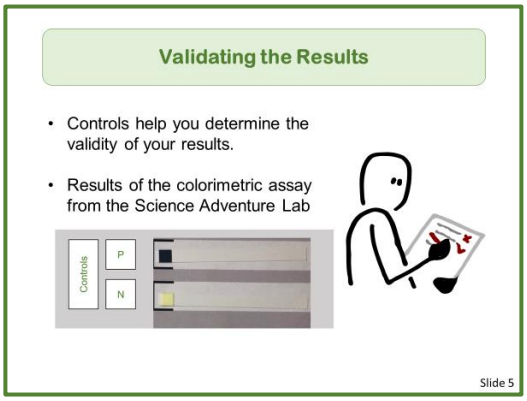
Key Vocabulary
- **Color scale**
- **Concentration**
- **Control**
- **Standards**

Materials Needed
- If students have an existing science lab notebook or composition book, it is recommended they use that to show their work. Loose paper could be used as an alternative.
- PowerPoint presentation with videos for Lesson 3.
- Teacher manual.
- Pencils.

SAMPLE

Slide 5 | Lesson 3 | *2 minutes*

Slide 5



Validating the Results

5A. Congratulate students on completing the assay. Tell students that before scientists share their results, an important step is to validate the results (or make sure they are accurate).

5B. Tell students one important part of making sure that your results are valid and that your experiment worked the way you expected is to use controls. All good science experiments include controls.

5C. Review with students the results of the colorimetric assay performed in the Science Discovery Lab.

- The positive control contained 2,800mg/L of Vitamin C and caused a test strip to turn a very dark green or almost black color. The positive control shows us what to expect when the substance is present.
- The negative control (water) contained no Vitamin C and caused a test strip to not change color. The negative control shows us what to expect when the substance is absent.
- These controls indicate to us that our assay is valid. This gives us the confidence that our standards, unknowns, and the test strips will yield valid results.

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