



Antioxidants and Cancer

Overview:

In this series of activities students consider the relationships among antioxidants, free radicals, and cancer. They perform a laboratory experiment to analyze the antioxidant concentrations in several types of beverages. Then they read an advertisement for a nutritional supplement and design a large scale research study to test the claim that this supplement prevents cancer.

Note: An asterisk (*) indicates activities that could be done as homework.

Part 1: Eat Your Fruits and Vegetables! (20 minutes) *

Students explore the relationships among antioxidants, free radicals, and cancer by reading an article and answering questions.

Part 2: Comparing Antioxidant Properties of Beverages (80 minutes)

Students perform a laboratory activity in which they:

- Use known concentrations of antioxidants to prepare a standard curve graph for tests to measure antioxidant concentration.
- Determine and compare the antioxidant concentration in five different beverages provided by the teacher.

Part 3: Evaluating Claims for Antioxidant Supplements (20 minutes) *

Students read an advertisement for a nutritional supplement containing antioxidants. They design a large-scale research study to test the claim that this supplement prevents cancer.

Part 4: Antioxidant Inquiry (80 minutes)

Students apply their understanding of antioxidant testing to design, conduct, and report on an experiment to answer a research question about antioxidant concentrations posed by their team or by the teacher.

Part 1: Eat Your Fruits and Vegetables! (20 minutes)

Students read “Part 1: Eat Your Fruits and Vegetables” and answer questions about the relationships among antioxidants, free radicals, and cancer. This activity may be done as homework or during class.

- If done during class, this activity might be preceded by asking students to create a concept map that shows how they think the words in the following list are related.
 - Antioxidants
 - Cancer
 - Free radicals
 - DNA
 - Mutation
 - Fruits
 - Vegetables

- Distribute one copy of “Part 1: Eat Your Fruits and Vegetables” to each student.
- Ask students to read the article and answer the questions.

Part 1: Eat Your Fruits and Vegetables!

Some research studies have suggested that a diet rich in fruits and vegetables may lower people's risk of cancer. Why? Because fruits and vegetables contain chemicals called antioxidants. Typical antioxidants include many phytochemicals (chemicals produced by plants) such as vitamin C (found in citrus fruits), carotenes (orange plant pigments), and anthocyanins (red or blue plant pigments).

Scientists believe that antioxidants may protect cells from damage caused by unstable molecules known as free radicals. Free radicals are atoms or molecules that are highly reactive because they have at least one unpaired electron. Free radicals may damage DNA molecules and lead to mutations that cause cancer. Free radicals are formed in your body by normal chemical reactions such as the breakdown of amino acids and fats. Exposure to environmental factors, including tobacco smoke and radiation, can also lead to free radical formation.

Antioxidants are chemicals that prevent free radicals from being formed and remove them before they do any damage. Cell culture and animal research has shown that antioxidants prevent free radical damage that could lead to cancer. However, research studies done in the 1990's to investigate the effects of antioxidants in preventing human cancer were inconclusive. Scientists are currently conducting three large-scale clinical trials to determine whether there is evidence to support the claim that a diet high in antioxidants reduces a human's risk of getting cancer.

1. What is a free radical?
2. How are free radicals formed?
3. How could an excess of free radicals lead to cancer?
4. What is an antioxidant?
5. What are phytochemicals?
6. What antioxidant advertising have you seen on TV or in magazines? What products were these advertisements trying to convince you to buy?
7. List five foods that you eat that you think (or have heard) are high in antioxidants.
8. How might a diet rich in antioxidants act to reduce a person's risk of cancer?
9. Predict how an increase in exercise would affect an individual's free radical concentration.
10. A diet high in antioxidants does not eliminate a person's chance of developing cancer. How could you explain this observation?

Part 2: Comparing the Antioxidant Properties of Beverages (80 minutes)

Students work in teams of 5 students to perform a laboratory activity in which they:

- Use known concentrations of antioxidants to prepare a standard curve graph for tests to measure antioxidant concentration.
- Determine and compare the antioxidant concentration in five different beverages provided by the teacher.

Lab Supplies distributed to each student:

- 1 copy of “Part 2: Comparing the Antioxidant Properties of Juices”
- 1 plastic well plate—wells at least 2 mL capacity
- 1 graduated plastic pipet—labeled “Known Antioxidant Solution”
- 1 graduated plastic pipet—labeled “Beverage”
- 1 graduated plastic pipet—labeled “Starch Suspension”
- 1 plastic thin stem pipet (Beral)—labeled “Iodine”
- 4 toothpicks
- 1 sheet of white paper
- 1 pair of goggles

For cleaning droppers and pipets, students will need access to water (from tap or a bucket) and paper towels.

Known Antioxidant Solutions:

Make a stock 5 mg/mL solution of antioxidant by mixing one 500 mg Vitamin C tablet with 100 mL of distilled water. Use the chart below to prepare solutions of other known antioxidant concentrations. Pour 10 mL of each solution into small labeled plastic cups. Each team should get one cup of each antioxidant concentration.

Labels on Cups: Known Antioxidant Concentration (mg/mL)	Stock Solution mL	Distilled Water mL
5.00	200	0
2.50	100	100
1.25	50	150
0.50	20	180
0.00	0	200

Antioxidant Indicator:

Prepare 200 mL of antioxidant indicator by mixing 0.008 grams (or 1/4 teaspoon) of DPIIP (diphenol indophenol) with 200 mL of distilled water. Filter before using. Each team should get one plastic cup containing about 10 mL of the DPIIP solution. Label cup “Antioxidant Indicator.” Team members will share the cup of indicator solution.

Beverages:

Select five of the beverages from the list below or substitute similar beverages. If substituting other beverages, select ones that are not highly colored. Pour 10 mL of each beverage into small labeled plastic cups. Each team should get one plastic cup of each type of beverage.

- Apple juice
- Cider
- Green tea
- Black tea
- Orange juice
- White grape juice
- Colorless Gatorade

Part 2: Comparing the Antioxidant Properties of Beverages

The concentration of antioxidant in different beverages varies. Examine the five samples of beverages provided by your teacher.

- Which beverage do you think has the highest concentration of antioxidant? _____
- Which beverage do you think has the lowest concentration of antioxidant? _____

Your Lab Team's Tasks:

In this lab activity you will test the five beverages to determine and compare their concentrations of antioxidants. You will:

- Use known concentrations of antioxidants to prepare a standard curve graph.
- Use the standard curve graph to determine the antioxidant concentration for each beverage.
- Compare the antioxidant concentrations in the five beverages.

A. Preparing a Standard Curve for Antioxidant Concentration

1. Assign each member of your team to test a solution with a different known antioxidant concentration shown in second column of Table 1. Write the initials of the team members responsible for each concentration in the first column of Table 1.

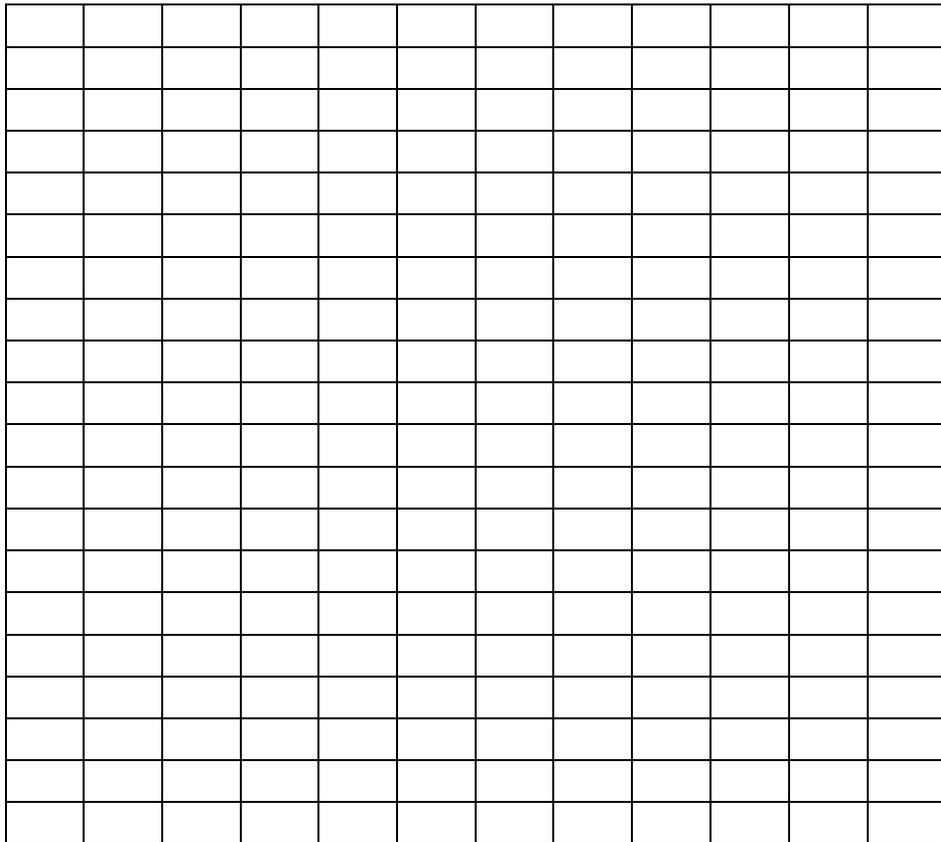
Table 1. Team Data for Solutions with Known Antioxidant Concentrations

Team Member's Initials	Antioxidant Concentration of Known Solution (mg/mL antioxidant)	Average number of drops of Antioxidant Indicator required to turn the mixture dark blue.
	5.00	
	2.50	
	1.25	
	0.50	
	0.00	

2. Use the known antioxidant solution assigned to you. Use a graduated pipet to add 0.25 mL of this antioxidant solution to one well of a clean well plate.

3. Place a sheet of white paper underneath the well plate.
4. Add one drop of Antioxidant Indicator to the well that contains the known antioxidant concentration you are testing. Use a toothpick to gently stir the mixture for 20 seconds or until the solution turns dark blue. Note: The solution may turn blue but may change back to the original color as you stir.
5. Carefully keep track of the number of drops of Antioxidant Indicator you use. Repeat step 4 until the mixture in the well remains dark blue for 20 seconds. Count and record the number of drops of Antioxidant Indicator needed to turn the mixture dark blue. **Number of drops** = _____
6. Do a second trial by repeating steps 2 through 5 using a clean well on the well plate and a clean toothpick. Use the same antioxidant solution that you used for your first trial. Record the number of drops of Antioxidant Indicator needed for the second trial. **Number of drops** = _____
7. Calculate and record (in Table 1) the average number of drops of Antioxidant Indicator needed to turn the mixture dark blue.
8. Complete Table 1 by sharing and recording the results with your team members.
9. Prepare a **line graph** (see next page) to represent the team data in Graph 1. Put concentration of antioxidant (mg/mL) on the horizontal (X) axis and the number of drops of Antioxidant Indicator needed to turn the mixture blue on the vertical (Y) axis.
10. State one conclusion based on the information in your graph.

Graph 1: Standard Curve for Antioxidant Test



B. Testing the concentration of antioxidant in the five beverages

Your team will now determine the concentration of antioxidant in the five types of beverages.

1. Assign each member of your team to test a different beverage. Record the initials of each team member in the appropriate space in the first column of Table 2.

Table 2: Team Data for Various Beverage Samples

Team Member's Initials	Beverage Name	Average number of drops of Antioxidant Indicator required to turn the beverage dark blue	Antioxidant concentration (mg/mL)

2. Repeat the procedure from Part A (steps 2-8) using the beverage you are testing instead of a known antioxidant solution. Record the number of drops of Antioxidant Indicator required to turn the beverage dark blue.
3. Do a second trial by repeating the testing using a clean well on the well plate. Record the number of drops of Antioxidant Indicator needed to turn the beverage/starch solution blue-black for the second trial. **Number of drops = _____**
4. Wash and dry the well plate. Rinse the droppers. Return the cups of solutions to your teacher.
5. Calculate and record the average number of drops of Antioxidant Indicator on Table 2.
6. Complete the third column of Table 2 by sharing and recording the results with your team members.

Analysis Questions:

1. Which beverage had the highest antioxidant concentration? _____
2. Which beverage had the lowest antioxidant concentration? _____
3. How reliable to you think the antioxidant testing was in determining the concentration of antioxidants in the five beverages? Rate the reliability of the antioxidant testing by marking an X on the scale below.

Very reliable

Somewhat reliable

Not reliable

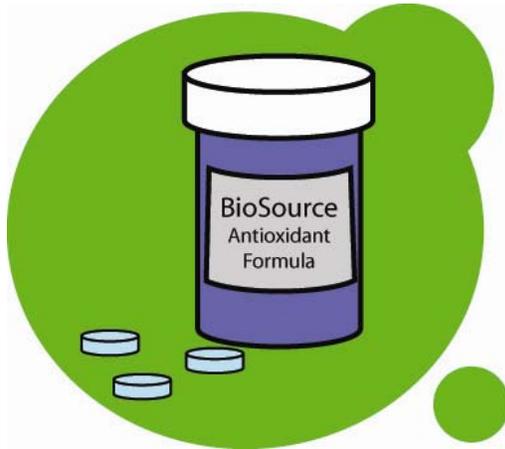
4. What are three possible sources of error for the antioxidant testing that your team did? Do not say "human error." Refer to the steps in the procedure.

Part 3: Evaluating Claims for Antioxidant Supplements (20 minutes)

Students read "Part 3: Evaluating Claims for Antioxidant Supplements", an advertisement for a nutritional supplement containing antioxidants. They design a large-scale research study to test the claim that this supplement prevents cancer. This activity may be done for homework or in class.

Part 3: Evaluating Claims for Antioxidant Supplements

Directions: Read this advertisement for a nutritional supplement that contains antioxidants. Then, answer the questions at the end of the advertisement.



Prevents CANCER

Ideal for:

ATHLETES, CANCER & AIDS PATIENTS

BENEFITS OF USE:

Free Radicals are unstable molecules generated as a by-product of breathing, causing a chain reaction, eating away at cellular membranes and damaging the genetic material. Antioxidants counteract the effects of free radicals. They help your body by stabilizing free radicals before they harm the cell.

DOSES & DIRECTIONS:

Adults take 1 capsule per day, preferably at meal time or as directed by physician.

INGREDIENTS:

Nutrition Facts: Serving Size 1 Capsule

Vitamin A (Beta Carotene) 10,000 IU.	200%
Vitamin E (Natural d-alpha) 200 IU	666%
Vitamin C (with rosehips) 250 mg.	416%
Zinc 10 mcg.	66%
Selenium 25 mcg.	none

* Percent Daily Values are based on a 2,000 calorie diet.

Bio Source

ANTIOXIDANT Formula

All Natural

FREE RADICAL NEUTRALIZER

Win the war against cancer in YOUR body. Use the power of antioxidants to fight cell damage caused by free radicals!

The Bio Source Antioxidant Formula provides 2-6 times the RDA for vitamin A (beta-carotene), vitamin C and vitamin E with selenium & zinc.

1. Do you think this dietary supplement is effective in preventing cancer? Explain why or why not.

2. The advertisement claims that the Bio Source Antioxidant Formula prevents cancer. Describe a **large-scale** investigation (involving a large number of subjects) that could be used to determine if the claim in this advertisement is valid. In your answer, include at least a description of:
 - The hypothesis that you will be testing in your research study [1 credit]
 - The treatment given to the experimental group [1 credit]
 - The treatment given to the control group [1 credit]
 - The dependent variable [1 credit]
 - The independent variable [1 credit]
 - Three factors that must be kept the same in both the experimental group and control group [3 credits]
 - The type of data which should be collected to support or refute the hypothesis [1 credit]
 - When the data should be collected [1 credit]
 - A data table to organize the results of the experiment [1 credit]
 - An example of experimental results that would lead to the conclusion that the claim was valid [1 credit]
 - Two reasons why doing this type of research study would be difficult [2 credits]

14 credits total

Part 4: Antioxidant Inquiry (80 minutes)

Distribute one copy of “Part 4: Antioxidant Inquiry” to each team of students. Students work in teams to design, conduct, and report on an experiment to answer a research question about antioxidant concentrations posed by their team or by the teacher.

Part 4: Antioxidant Inquiry

Here are some examples of questions that people might ask about antioxidant concentrations in their foods.

- Which type of chocolate (dark, semi-sweet, or milk) contains the greatest concentration of antioxidants?
- Which type of vegetable (broccoli, spinach, or green beans) contains the greatest concentration of antioxidants?
- Which type of tea (green, herbal, or brown) contains the highest concentration of antioxidants?
- How does boiling, microwaving, and freezing foods affect their antioxidant concentrations?

Design, conduct and report on an experiment to determine the answer to one of these questions OR, better yet, to a question about antioxidant concentrations in foods that your team is curious about.