



CAROLINA[®]
Teamed with Teachers

Teacher Demo
17-3603

3-Station Kit
17-3603A

6-Station Kit
17-3603B

Sunscreen for Yeast and People, Too

Teacher's Manual

Objectives

This kit is designed to

- Heighten the student's appreciation of the damaging effects of the ultraviolet light found in sunlight.
- Demonstrate to the students the importance of DNA repair in protecting organisms from ultraviolet light.
- Familiarize the students with the scientific method.
- Make the students aware that simpler organisms can be used to help understand processes in more complex organisms.

Overview

In the first part of this lab, students will use the scientific method to test the difference in sensitivity of two yeast strains to ultraviolet light. One yeast strain is wild-type, and the other is a strain that is unable to effectively repair damage to its DNA. The student's findings should demonstrate that the yeast cells that are unable to repair their DNA are extremely sensitive to sunlight.

In the second half of the lab, students will test the effectiveness of various sunscreens and cloth in preventing ultraviolet-light damage to the ultraviolet light-sensitive yeast. Students are encouraged to think about the implications of the results of these labs with respect to the effect that exposure to the sun and other sources of ultraviolet light have on the human body and on other higher living organisms.

Materials

Included in the kits

Demo	3-station	6-station	
17-3603	17-3603A	17-3603B	
1	1	1	wild-type yeast strain on a YEPAD slant (store refrigerated at 4°C)
1	1	1	UV-sensitive yeast strain on a YEPAD slant (store refrigerated at 4°C)
1	3	6	box of sterile toothpicks
4	10	18	sterile, capped plastic test tubes
1	2	3	sleeve of 20 petri dishes

Demo	3-station	6-station	
17-3603	17-3603A	17-3603B	
1	3	4	bottle YED media (350 mL)
5	10	19	1-mL individually-wrapped sterile bulb pipets
3	7	13	5-mL individually-wrapped sterile bulb pipets
1	3	6	bottle of sterile, distilled water (20 mL)
1	3	6	small jar of glass beads
1	1	1	photocopy master for Student Guide
1	1	1	Teacher's Manual

Needed, but not supplied

- aluminum foil or other opaque material to shade one-half of petri plates
- 2 or more types of sunscreen
- 14 × 7-cm pieces of commonly worn fabric (i.e., knit cotton like that in T-shirts)
- plastic wrap
- 30°C incubator (optional)

Background

We are all familiar with the damaging effect that sunlight has on our own skin, especially when we are overexposed to it. However, most of us are less familiar with the effect that sunlight has on other organisms and how they can be used to study the effect of overexposure to the sun on our own bodies.

This kit discusses and demonstrates the effect of sunlight on yeast, an organism frequently used as a model for studying mammalian biology. This material also explains why studying the effect of sunlight on yeast is useful to scientists and, ultimately, to all of us. In this lab you will use a bioassay to compare the effectiveness of various sunscreens and types of cloth in shielding UV light-sensitive yeast cells from the damaging effects of the sun. Performing this assay should enhance your understanding and appreciation of why overexposure to sunlight is harmful.

Sunlight is a form of energy composed of many types of electromagnetic radiation, including visible and ultraviolet (UV) light. As do all forms of electromagnetic radiation, visible and UV light travel in waves. Visible light, which includes all the colors of the rainbow (red, orange, yellow, green, blue, indigo, and violet), has wavelengths in the range of 760–400 nm (a

nanometer (nm) is 1×10^{-9} m). In contrast, UV waves are shorter (less than 400 nm). Ultraviolet light is often divided into three categories, UV-A (400–320 nm), UV-B (320–290 nm), and UV-C (290–180 nm). The ozone layer, a protective layer of O₃ in the earth's upper atmosphere, absorbs all of the UV-C light and most of the UV-B. Thus, only UV-A and some UV-B light reach the surface of the earth. Sunscreen is designed to protect us from these remaining UV light waves.

In addition to traveling in waves, light comes in particles called photons. The shorter the wavelength of light, the more energy a photon of that light has. For example, blue light (480–450 nm) has a shorter wavelength than red light (760–610 nm), so photons of blue light are more energetic than photons of red light. Ultraviolet light waves (less than 400 nm) are shorter than blue, red, or any other visible light. Thus, photons of UV light are even more energetic than those of visible light. Because of the high energy of its photons, UV light can disrupt chemical bonds, including the bonds that make up the cells of living organisms.

DNA, the chemical in our cells that is essential for controlling proper growth and function, is routinely damaged by our daily exposure to UV light. DNA-coded proteins in our cells control cell growth and function. UV light damage to DNA can change protein structures, causing a cell to become cancerous or to die.

Some of the most significant UV-induced damage to DNA is the linkage of adjacent DNA base pairs to form dimers. These abnormal dimers distort the DNA helix and block its transcription into RNA and its replication by the DNA copying machinery. One dimer per cell can be lethal.

To protect themselves from cancer or death, cells have evolved special enzymes (types of proteins) to repair DNA damage caused by UV light. However, occasionally, the cell's repair enzymes make a mistake that results in a mutation, a change in the DNA sequence. Exposure to UV light increases the degree to which a cell must repair its DNA, increasing the chance of mutations from faulty repair.

As mentioned before, the upper atmosphere (stratosphere) contains enough ozone to filter out most, but not all, of the damaging UV photons. The small percentage of UV light that reaches the earth is still capable of doing a considerable amount of damage to living organisms. It can still give you a sunburn, skin cancer, or eye damage if you are exposed to it for too long. Even tanning, the skin's production of the pigment melanin in response to sun exposure, is accompanied by some DNA damage.

People with the genetic disease xeroderma pigmentosum are especially sensitive to ultraviolet light. This sensitivity results from their not being able to repair their DNA as efficiently as most. Not surprisingly, people affected by this disease have a very high rate of cancer.

The lens of our eye is particularly vulnerable to damage by sunlight. UV absorbed by the proteins that make up the lens of the eye contributes to the formation of cataracts. Cataracts decrease the ability of the lens to transmit light and cause partial or complete blindness if the lens is not surgically replaced with an artificial one.

However, some exposure to UV light is necessary for good health. For example, we can only make vitamin D when solar UV light stimulates our skin. Since vitamin D is essential for normal calcium and phosphorous metabolism, if we do not get enough sunlight to make vitamin D, we have to add it to our diet. Otherwise, the lack of vitamin D causes rickets, a disease marked by weak and malformed bones.

The amount of time you can safely be exposed to sunlight depends on several variables:

- **Clouds and haze.** Clouds and haze in the lower atmosphere (troposphere) absorb some UV photons.
- **The angle of the sun.** When the sun is directly overhead the most UV gets through the atmosphere. When the sun is lower in the sky—in the winter, early or late in the day, and in places far from the equator—less UV light gets through the atmosphere because it must travel through more of the atmosphere. The more atmosphere the photons must travel through, the greater the chance that their energy will be absorbed before they reach the earth's surface.
- **Altitude.** At higher altitude there is more UV light not because there is less ozone, but because there is less haze.
- **Shading.** Shading the skin with clothing or anything else dramatically reduces or cuts off exposure to UV light.
- **Other absorbent material.** Glass and most plastics absorb UV photons. UV light-absorbing films can be put on the surface of glass and plastics.
- **Skin pigment.** Melanin, the skin pigment present to varying degrees in our skin, absorbs UV light and helps protect dark-skinned people.
- **Sunscreen.** Sunscreens contain chemicals that absorb UV photons.

Given how dramatically ultraviolet light can affect living organisms, it is easy to understand how critical the protective ozone layer around the earth is to the well being of many organisms. In recent times, for reasons which are not completely understood, the ozone layer has been thinning. This is

believed to be associated with human-generated pollutants and has caused much concern and debate within the scientific and political communities.

Scientists like to use a good “model system” for many of their studies. A good model system or model organism serves as a good representation of the actual organism the scientist would like to study. However, the model organism is simpler and easier to study than the actual one. Yeast cells are a useful “model system” to study the effects of UV light on higher organisms for several reasons. Yeasts are single-celled and easy to grow, so they are simpler and easier to study than a complex organism like a human being or other mammal. However, like mammals, yeasts are eukaryotes; thus, their DNA is organized and replicated in much the same way as mammalian DNA. Finally, the enzymes yeast cells use to repair their DNA are very similar to the enzymes used in mammalian cells. Studying the DNA-repair enzymes in yeast cells has been critical to understanding human DNA-repair enzymes.

In this activity, you will use UV-sensitive yeast cells to measure the lethal effect sunlight can have on an organism if it is not able to repair its DNA. Dr. John Game developed this yeast strain by introducing mutations into the genes necessary for the repair of DNA. Observing how sensitive these yeast cells are to sunlight will give you an idea of the degree of damage that our cells must repair every day in response to our exposure to the sun.

In the first part of this kit, you will expose a yeast strain that is deficient in DNA repair and a wild-type strain to sunlight to observe the effect the mutations in the DNA-repair enzymes have on the yeast cells’ ability to tolerate exposure to sunlight. The wild-type yeast cells will be included in the experiment as a control. Because the repair enzymes of the wild-type yeast cells are intact, the wild-type cells should tolerate exposure to the sun to a greater degree than the UV-sensitive mutants.

In the second part of the lab, you will use the UV light-sensitive yeast strain to determine the protective effect of various sunscreens and types of cloth. For this experiment, your teacher will ask you to bring in sunscreen or tanning oil, and a piece of cloth, such as part of an old T-shirt.

Timeline

Preparation for Labs

1. Pour YED plates (plates may be poured anywhere from 2 weeks to 1 day prior to their use.)
2. Prepare 2 master plates (one of each yeast strain). Do this 1 day prior

(if you are growing the cells at 30°C) or 2 days prior (if you are growing the cells at room temperature) to preparing plates for student stations.

3. Streak yeast strains from master plates onto YED plates (one plate of each strain for every two lab stations). Do this 1 day (if using 30°C incubator) or 2 days (if growing the yeasts at room temperature) prior to the actual lab.

Lab Procedures

Lab 1. 45 minutes. Plate the wild-type and UV-sensitive yeast strains and expose them to UV light.

Lab 2. 30 minutes (1 or 2 days after Lab 1). Observe the growth and discuss the results.

Lab 3. 45 minutes. Plate the UV-sensitive yeast strain and expose it to the sun in the presence and absence of various UV light blocking agents.

Lab 4. 30 minutes (1 or 2 days after Lab 3). Observe the growth and discuss the results.

Note: If you grow the yeast cells at room temperature, allow 2 days for them to grow in the steps that follow.

Pre-Lab Setup

Note: Always protect the UV light-sensitive yeast strain from light when you are not working with it.

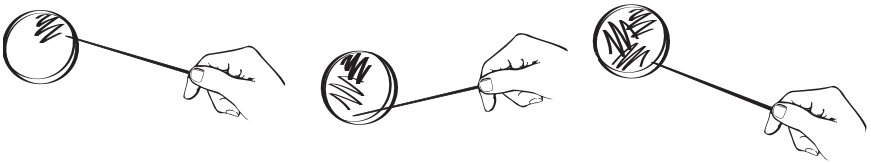
Pouring YED Plates

1. Make a clean workspace by wiping down your work area with ethanol or soapy water.
2. Loosen the cap of the bottle of YED agar media and heat the bottle in either a microwave oven (5–10 minutes), or a boiling water bath (20–30 minutes) until the agar is completely melted. To prevent the YED agar media from boiling over, swirl the bottle every couple of minutes during heating. Pour just enough melted YED agar media into each petri dish to cover the bottom of the dish (20–22 mL for each plate). Remove the lid of the dish just long enough to pour the YED agar media. Replace the lid immediately after you finish pouring. Extra petri dishes are included with the kit; therefore, you will not have enough media to fill all the petri dishes. Use a padded glove to protect your hands during these steps. When the plates have cooled, solidified, and

any condensation on the lids has dried, put them back into the original sleeve or in a resealable bag and store them at 4°C in a refrigerator. After pouring, the plates may be left out one night at room temperature. Since plating the yeast is easier on plates that do not contain an excessive amount of moisture, you may wish to make the plates several days ahead of using them.

Streaking Master Plates

1. Make a clean workspace by wiping down the work area with ethanol or soapy water.
2. The slant that comes with the kit is made from YEPAD medium, a rich medium used to store yeast. Streak a single master plate for both the wild-type and the mutant yeast strain by using the toothpicks included with the kit. Grasp a toothpick in the center to remove it from its box. Avoid touching the other toothpicks as much as possible, and do not touch the end you will use to pick up the yeast. Streaking a master plate for each strain allows you to preserve the original culture so that you may use it for any future experiments.



Streak each plate by touching a sterile toothpick to an area where yeast growth is apparent. If there is a single colony that you can touch, that is ideal, because it will give you a pure culture. Glide the toothpick across the YED plate in a zig-zag pattern. Use a fresh toothpick to create another zig-zag pattern, starting at and running through the original streak. Get a fresh toothpick and use the second pattern as the starting point to make a third pattern (see figure above). The yeast cells that grow in the track of the second or third pattern should be distributed enough so that you will be able to isolate single colonies to streak the student starter plates from. Allow these two master plates (one containing wild-type yeast cells and one containing the UV light-sensitive yeast cells) to grow overnight at 30°C or at room temperature for a couple of days. Do not forget to label your plates appropriately.

Streaking Starter Plates

One or 2 days before the first lab session (depending on the temperature at which you grow the yeasts), use the master plate to streak one starter plate

with the UV light-sensitive yeast strain and another starter plate with the wild-type yeast strain. Make one starter plate of each strain for **every two** student stations. Pick a single colony to streak each plate and use the same technique you used to streak the master plates. Be sure to label the plates as either wild-type or mutant. Make sure you do this 1 day (if you are growing the yeast cells at 30°C) or 2 days (if you are growing the yeast cells at room temperature) prior to the first lab session. This will ensure that fresh cultures are used for each experiment. **You will need to streak new starter plates 1–2 days prior to the second lab session.**

Setting Up the Student and Central Workstations for Lab 1

Place the following materials at each student workstation:

- 2 YED plates
- bottle sterile water
- 2 squares of aluminum foil, 12 × 12 cm (not included in kit)
- 2 sterile, capped, plastic test tubes
- 2 1-mL individually-wrapped sterile bulb pipets
- 5-mL individually-wrapped sterile bulb pipets
- small jar of glass beads
- box of sterile toothpicks
- starter plate with the UV light-sensitive yeast strain (**shared between two stations**)
- starter plate with the wild-type yeast strain (**shared between two stations**)

Place the following items at the central, shared workstation:

- 2 timers or watches that can be taken outside for timing the exposure of the yeast to the sun.
- 30°C incubator (optional)

Presenting the Lab to Your Students

For the first lab period, supply the students with the minimal amount of background. Tell them that one yeast strain is wild-type and that the other yeast strain is not able to efficiently repair damage to its DNA. Explain that this experiment is to figure out how the two strains each respond to exposure to the sun. Ask the students what they think will happen (i.e., their hypotheses) and why they think so. **Do not supply the students with the introductory material until after they have drawn their own conclusions from the results of the first lab exercise.**

Lab Period 1

Procedure

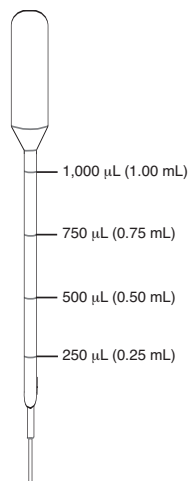
Making a yeast cell suspension

Maintain sterility as much as possible in this procedure.

1. Label the two tubes at your workstation as follows: *WT yeast strain*, *Mutant yeast strain*.
2. Use one of the sterile toothpicks at your station to collect a mass of yeast from the starter plate labeled *WT yeast strain*. Pick the toothpick out of the box from the center of the toothpick. Avoid touching the other toothpicks as much as possible and do not touch the end of the toothpick that you use to pick up the yeast. The mass of the yeast on your toothpick should be about 1 mm in diameter. Smear the mass on the inside surface of the tube as far down as you can easily reach. Using a clean toothpick and the appropriately labeled tube, repeat the procedure with the mutant strain.
3. Use one of the sterile, plastic, 5-mL bulb pipets to pipet 5 mL of sterile water into each of the yeast-containing tubes on your bench. To avoid cross contamination, make sure that you do not touch the walls of the tubes with the pipet. **When using bulb pipets, maintain sterility by opening the pipet packet from the bulb end (the side away from the end used to take up liquid).**
4. Shake or vortex the tubes until the yeast cells are completely re-suspended in the water. The suspension should be slightly cloudy.

Plating the yeast cell suspension

1. Label one of the plates on your bench *WT strain* and the other *mutant strain*. With the lid-side down, open the plate just long enough to shake 4–5 glass beads onto the lid of the plate. Close the plate and flip it back over.
2. Use the 1-mL bulb pipets included with the kit to pipet 250 μL of each yeast suspension onto the appropriately labeled plate (see diagram for the location of the 250 μL mark on the 1 mL bulb pipets.) **Make sure you use a different pipet for each strain.** Maintain sterility as much as possible.
3. Spread the yeast cells onto the plate by shaking the glass beads back and forth across the **entire surface**

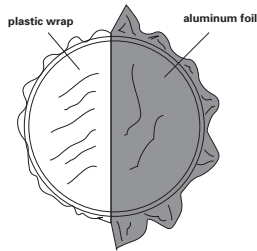


of the plate (not in a swirling motion, which will only run the beads around the edge of the plate).

4. When you are finished spreading, let the plate sit until the excess liquid has soaked into the agar.
5. To remove the glass beads, hold each plate vertically over a container and open the plates slightly (like you are opening clamshells) to allow the beads to drop into the container.

Exposing the yeast cell suspension to ultraviolet light

1. One at a time, remove the lid from each of the plates you spread yeast on and quickly cover the plate with plastic wrap. Pull the wrap taut and make sure it does not touch the surface of the plate. Since some petri dishes contain a pigment that absorbs some components of sunlight, it is preferable to use the plastic wrap in place of the petri dish lid when exposing the yeast cells to the sun. Use the squares of aluminum foil to cover half of the yeast plates so they will be shielded from the sun. The plates should look like this:



2. Expose the plates to the sun according to the information given in the following table. To maximize exposure, make sure that the surface of each exposed plate is perpendicular to the sun during the exposure. The shadow that the plate casts on the ground is at its smallest when the plate is in this position.

	Midmorning	Noon	Midafternoon
Summer	15-20 minutes	10-15 minutes	15-20 minutes
Spring and Fall	25-30 minutes	15-20 minutes	20-25 minutes
Winter	200-250 minutes	75-100 minutes	100-150 minutes

Adapted from *A Classroom Guide to Yeast Experiments* by the Genetics Education Network, Kansas State University.

3. After you have exposed the plates, remove the plastic wrap and quickly replace the lids. Incubate the plates overnight at 30°C or for 48 hours at room temperature.

Lab Period 2

Results and Analysis

Examine your incubated plates.

1. In a few sentences, describe what you see on your plates. Estimate what percentage of cells has been killed on each of your plates.
Students should find that the growth on the plate containing the wild-type yeast is relatively unaffected by exposure to the sun. On the plate containing the UV light-sensitive yeast there should be reduced or no growth on the side of the plate exposed to the sun. The degree of the reduction in growth will depend on the length of exposure time and the intensity of the sun on the day of exposure (which is affected by the altitude and latitude as well as the extent of haze or cloud cover).
2. Which yeast strain has been affected more by the sunlight?
Students should conclude that the mutant strain (the UV light-sensitive strain) has been affected more by sunlight.
3. Given what you know about these two strains, what role does a cell's ability to repair DNA play in how well it tolerates exposure to sunlight?
Students should come to the conclusion that DNA repair is important for a cell's ability to tolerate exposure to sunlight.
4. Why do you think it would be important for a cell to be able to efficiently repair its DNA in order to tolerate exposure to the sun?
From their conclusion in question 3, students should further conclude that cells are not able to function well if their DNA is severely damaged.
5. Why do you think exposure to sunlight is so damaging to yeast cells?
This question requires students to pull together all of their thoughts from the previous questions. Their answers should be somewhere along the lines of "Exposure to sunlight damages the yeast cells' DNA. If the DNA cannot be repaired, the cells may die."

Lab Period 3

Testing the Protective Effect of Sunscreen and Fabrics

Pre-Lab Setup

Place the following materials at each student workstation:

starter plate with UV light-sensitive yeast strain (shared between two stations)

4 YED plates

bottle of sterile water

square of aluminum foil, 12 × 12 cm

sterile, capped, plastic test tube

4 1-mL individually-wrapped sterile bulb pipets

5-mL individually-wrapped sterile bulb pipet

small jar of glass beads

box of sterile toothpicks

2 types of sunscreen or tanning oil (supplied by students)

pieces of common clothing fabric cut into squares, 14 × 7 cm
(supplied by students)

Place the following at the central, shared workstation:

2 timers or watches that can be taken outside for timing the exposure of the yeast to the sun.

30°C incubator (optional)

Procedure

Each group should bring in two types of sunscreen or tanning oil and a scrap of fabric that they want to test for its ability to block UV light.

To test the ability of the two different sunscreens and the piece of fabric to block ultraviolet light, you will need four plates containing the UV light-sensitive yeast strain:

- 1 control plate with one-half of it completely exposed and one-half completely protected by aluminum foil. The growth on the other three plates will be compared with the growth on this plate.
- 2 plates with one-half exposed and one-half protected with sunscreen.
- 1 plate with one-half of the plate exposed and one-half protected by fabric.

1. Prepare a yeast suspension as you did in Lab Period 1, but this time prepare only the mutant, UV light-sensitive yeast strain. In brief, use a toothpick to transfer a mass of yeast into a sterile tube and resuspend the yeast in 5 mL of sterile water.
2. Using the same technique as you did in Lab Period 1, plate 250 μL of the UV-sensitive yeast suspension onto each of the four YED plates. Use a fresh pipet for each plate. Be sure that you label each plate (e.g., *control*, *cotton T-shirt*, *Sans-a-Burn sunscreen*, *AloeHa sunscreen*).
3. Once the yeast has been plated and the excess liquid has soaked into the agar, remove the lids and cover the plates with plastic wrap as you did in Lab Period 1. On one plate, smear a thin layer of one of the sunscreens you are testing onto the plastic wrap over one-half of the plate. Smear the second sunscreen in a like manner over one-half of the second plate. Cover one-half of the third plate with the piece of cloth that you are testing. You may need to use clear tape to hold the cloth in place. Be aware that sunscreen looks clear as it dries. You may wish to draw a line on the bottom of the plate to mark the division between the protected and unprotected halves. Do not smear the sunscreen on or tape the cloth to the bottom of the plate.
4. Expose all three plates to the sun in the same manner as you did in Lab Period 1. Again, use the chart to determine the correct exposure time to use.
5. After exposing the plates to the sun, remove the plastic wrap, quickly cover the plates, and incubate them for 1 day at 30°C or 48 hours at room temperature.

Lab Period 4

Analysis

As a class, devise a way to score the extent to which the yeast cells are killed in the presence or absence of the various UV light-blocking agents tested. Remember to compare the amount of growth in the area protected by the sunscreen or cloth with the growth in the completely protected area of the control plate. The protected area on the control plate provides an indication of how much growth to expect, given the amount of yeast you plated.

Using the system you have devised, make a chart to report the degree to which the yeast cells are killed in the presence of the various blocking agents. The chart should include the result from the entire class. It should

also include the SPF (sun protection factor) of the different sunscreens and tanning oils, as well as descriptions of the cloth tested.

1. Do all of the sunscreens with similar SPFs perform equally well? If not, what reasons can you come up with to explain the differences?

Possible explanations included the following:

- *One sunscreen is older than the other.*
- *One sunscreen is more accurately represented by the manufacturer than the other.*
- *One sunscreen was applied in a much thicker layer than the other.*
- *There was variation in how the control plate and the experimental plates (the plates shielded by cloth or sunscreen) were treated within a working group. If students do not use a fresh pipet for each plate, or there is a pipetting error during plating, the plates will not have the same number of cells on them. This may lead to an inaccurate estimate of how many yeast cells are killed under the different conditions. Also, if the students are not careful about exposing the plates for the same amount of time and at the same angle to the sun, their estimates of how many cells are killed may be inaccurate.*

You may wish to ask your students what they could do to tell if the difference in supposedly similar sunscreens was a result of errors in how the assay was done or because of differences in the sunscreens themselves.

2. If yeast cells have DNA repair enzymes and DNA similar to our own, what do these results tell you about the degree of DNA repair that occurs in our own cells in response to our exposure to sunlight.
These results suggest that, like yeast, our DNA is damaged a lot by exposure to sunlight and that, as a result, a lot of DNA repair occurs in our cells in response to sunlight exposure.

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