

STUDENT ACTIVITY GUIDE – using colour chart

EQUIPMENT AND MATERIALS

Materials required by each student/group:

- 1 test-tube rack with 3 test tubes
- Marker pen
- Stop clock/timer
- Safety spectacles
- 1 syringe/Pasteur pipette

Materials to be shared:

- Yeast solutions-2.5% (live yeast and boiled yeast)
- 5% glucose
- 0.01% Resazurin dye
- Water bath at 35 °C
- Distilled water

INSTRUCTIONS

1. Collect the materials indicated above.
2. Label 3 test tubes A, B and C.
3. Add 3 cm³ of resazurin dye to each tube.
4. Add 3 cm³ of glucose solution to tubes A and C, then add 3 cm³ of water to tube B.
5. Add 3 cm³ of fresh yeast suspension to tubes A and B, then add 3 cm³ of boiled yeast suspension to tube C.
6. Gently shake each tube and place in a water bath at 35° C.
7. Record the colour of each tube every 3 minutes for 30 minutes by comparing tubes with the colour chart below.

The chart shows the colour changes you might expect as blue resazurin dye is gradually reduced. The dye should change colour as indicated from left to right in the chart.



The numerical values can be related to respiratory / dehydrogenase activity in yeast with 10 being equivalent to lowest activity.

STUDENT ACTIVITY GUIDE – using immobilised yeast and colorimeter

Cells such as yeast are often used in industrial processes. At the end of the process yeast cells remain mixed up with the product of the process and cannot be easily separated from it. Immobilisation is a method which traps the yeast cells in gel beads. Immobilised yeast is therefore more easily separated from the product. Immobilisation also means that the yeast can be re-used.

In this experiment immobilising the yeast makes it possible to separate the yeast from the dye and allows colorimetric measurements to be taken of each sample.

MATERIALS

- *3% sodium alginate solution (3 cm³)*
- *2% calcium chloride solution (30 cm³)*
- *2.5% yeast solution (3 cm³)*
- *10 cm³ syringe barrel*
- *Bijou bottles*
- *1 cm³ Pasteur pipette*
- *Plastic cup*
- *Tea strainer*
- *Stirring rod*
- *Retort stand*

INSTRUCTIONS FOR MAKING IMMOBILISED YEAST

1. Clamp the barrel of the 10 cm³ syringe barrel so that it is about 20 cm above the top of the plastic cup. Add the calcium chloride solution (30 cm³) to the plastic cup.
2. Gently shake the yeast in order to re-suspend the solution.
3. Add 3 cm³ yeast suspension to the 3 cm³ sodium alginate solution to a beaker and mix well using the stirring rod.
4. Pour the yeast/alginate mixture into the syringe barrel and gently swirl the plastic cup. As the mixture drops in to the calcium chloride, each drop will form a bead.
5. Once the syringe is empty leave the newly formed beads for approximately 5 minutes.

6. Transfer the beads to the tea strainer and gently wash them with cold tap water. Then rinse well with distilled water.
7. Beads can be used immediately or stored in distilled water until required.

INSTRUCTIONS FOR USING IMMOBILISED YEAST

Set up test tubes containing resazurin dye and glucose solution as for colour chart method but instead of adding yeast suspension to each tube, add 15 immobilised yeast beads. Set the colorimeter filter to 590nm, at this wavelength the disappearance of the blue colour of the resazurin dye is measured. Use the appropriate glucose solution to blank the colorimeter. Using a Pasteur pipette remove samples from each tube every 3 minutes and take colorimetric readings for 30 minutes, remembering to pour the solution back into the correct tube after each reading.

Further investigations into respiration rate in yeast using resazurin dye as an indicator:

- Type of yeast used (fresh, dried, frozen)
- Concentration of the yeast suspension
- Temperature at which the investigation is carried out
- Respiratory substrate (For example, sucrose / starch / maltose / fructose)
- Concentration of respiratory substrate

Further investigations into respiration rate using immobilised yeast and colorimeter readings:

- Any of the investigations above using the colorimeter method
- Compare respiration rates in immobilised and non-immobilised yeast

Investigating the activity of dehydrogenase in yeast using resazurin

TEACHER/TECHNICAL GUIDE

Materials required by each student/group:

- *1 test tube rack with 3 test tubes*
- *Marker pen*
- *Stop clock/timer*
- *Safety goggles*
- *1 syringe/Pasteur pipette*
- *Resazurin reduction colour chart*

Materials to be shared:

- *Yeast solutions 2.5% (live yeast and boiled yeast)*
- *Glucose or other carbohydrate solution (5%)*
- *Resazurin dye*
- *Water bath at 35 °C*

PREPARATION OF MATERIALS

Resazurin tablets can be bought from Scientific and Chemical at a cost of £22.49 for 100 tablets (July, 2016).

Dissolve one resazurin tablet in 25 cm³ of distilled water. Make up immediately before use. This will yield a solution which is approximately 1% w/v resazurin.

A wide variety of fresh, dried or frozen yeast can be used – use 2.5% solution.

For boiled yeast use 2.5% solution of chosen yeast, boil then allow suspension to cool before use.

Use 5% carbohydrate solutions (e.g. glucose/fructose/maltose/sucrose/starch/lactose).

If the activity is carried out as an investigation other requirements are:

- *Ice and apparatus for boiling water*
- *Additional 100 cm³ beakers and syringes for carrying out serial dilutions*
- *A variety of different types of yeast such as bery dried yeast, distillers yeast, wine yeast, autolysed yeast etc*

If using immobilised beads and colorimeter, students will also need:

- *Colorimeter set at 590 nm*
- *3% sodium alginate solution*
- *2% calcium chloride solution*
- *Bijou bottles*
- *Plastic cups*

ADDITIONAL DATA SETS

Data sets generated using the associated student protocols are available on the SSERC website:

<http://www.sserc.org.uk/index.php/biology-2/biology-resources/higher-biology-revised/3976-data-sets-higher-biology-and-higher-human-biology>