

# Enzymatic activity of catalase with Cobra SMARTsense



Chemistry

Organic chemistry

Biochemistry

Biology

Biochemistry

Applied Science

Medicine

Biochemistry



Difficulty level

medium



Group size

-



Preparation time

10 minutes



Execution time

45+ minutes

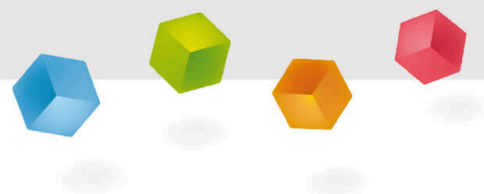
This content can also be found online at:



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## General information



## Application

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Experimental setup

Catalase is an enzyme that is excellently suited for demonstrating enzyme activity under various conditions. For this experiment, we detect the pressure that is generated when hydrogen peroxide is split into water and oxygen. We use the Cobra SMARTsense Absolute Pressure for this purpose.

## Other information (1/5)

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### Prior knowledge



Students should be familiar with the topics of cellular respiration (and cellular respiratory toxin), the influence of hydrogen peroxide on the body and enzyme function.

### Principle



The enzymatic degradation of the cellular respiratory toxin hydrogen peroxide in the liver can be measured very elegantly via the increase in pressure in an airtight reaction vessel, because the enzyme catalase forms hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) Oxygen (and water).

## Other information (2/5)

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### Learning objective



The students should realise that oxygen and water are formed in the liver when hydrogen peroxide is broken down. This can be demonstrated by an increase in pressure.

### Tasks



The students investigate the enzymatic degradation of hydrogen peroxide in the liver. They also investigate the influence of temperature and pH on metabolic activity.

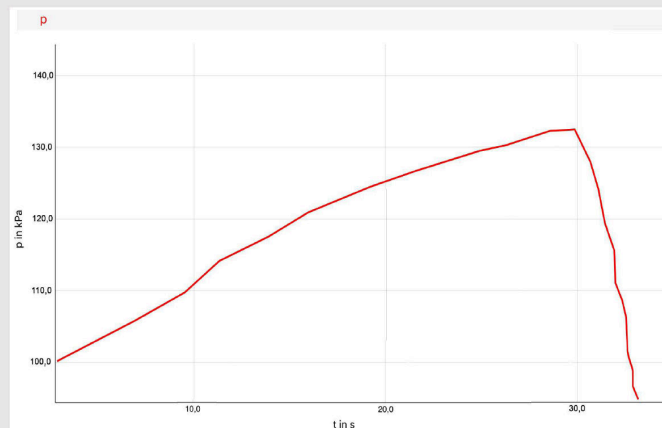
## Other information (3/5)

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### Observations and results

**Experiment 1:** In the first experiment (no addition of reagents, standard temperature) a steep increase of the pressure curve can be seen (figure right).

In the course of the measurement, the curve drops vertically because the rubber stopper was pressed out of the Erlenmeyer flask.



Measurement result normal

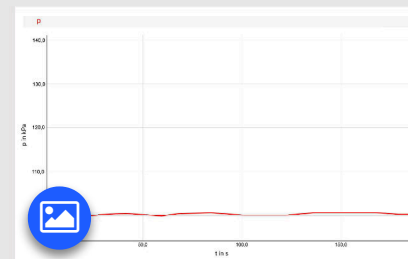
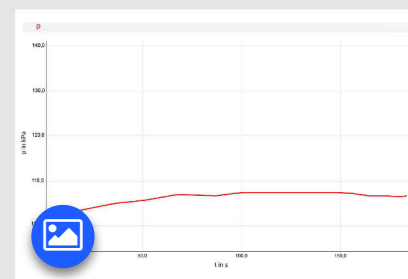
## Other information (4/5)

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### Observations and results

**Experiment 2a:** With the addition of **caustic soda** a smaller increase in the pressure curve can be seen compared to normal conditions (Fig. top right).

**Experiment 2b:** With the addition of **hydrochloric acid** no increase in the pressure curve can be seen (test below right).



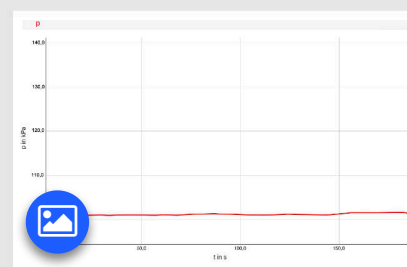
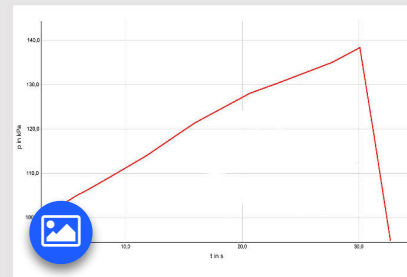
## Other information (5/5)

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### Observations and results

**Experiment 3a** After an approx. 5-minute **ice bath** the pressure rises almost as quickly as under normal conditions (Fig. top right). In the course of the measurement, there is a sudden drop in pressure, as here too the rubber stopper has been forced out of the Erlenmeyer flask.

**Experiment 3b** After an approx. 5-minute **heat treatment** the pressure in the Erlenmeyer flask remains constant (Fig. bottom right).



## Safety instructions

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- For the H- and P-phrases please refer to the corresponding safety data sheets.
- As considerable pressure is built up during the experiment, protective goggles should be used.
- The general instructions for safe experimentation in science lessons apply to this experiment.

## Theory

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Catalase is an enzyme that in humans occurs mainly in the liver and in erythrocytes. It breaks down hydrogen peroxide ( $\text{H}_2\text{O}_2$ ), a toxic by-product of cellular respiration, to water and oxygen. If, for example, blood is mixed with  $\text{H}_2\text{O}_2$ . This is how you see oxygen bubbles bubbling out.

Enzymes are dependent on the pH value. Catalase prefers the alkaline range. The enzyme reacts more sensitively to an acidic environment and is no longer active.

Enzymes consist of proteins. Proteins denature at high temperatures (catalase from approx.  $40^\circ\text{C}$ ). Therefore, in the heat experiment, there is no longer any increase in pressure after 5 minutes. The proteins of the enzyme were destroyed by the heat. A cold shock, on the other hand, inactivates the catalase only temporarily. After the temperature rises, the enzymes work normally again.

## Equipment

Position	Material	Item No.	Quantity
1	Cobra SMARTsense Absolute Pressure - Sensor for measuring the absolute pressure 20 ... 400 kPa (Bluetooth + USB)	12905-01	1
2	Support base, variable	02001-00	1
3	Support rod, l = 600 mm, d = 10 mm, split in 2 rods with screw threads	02035-00	1
4	Boss head	02043-00	2
5	Universal clamp	37715-00	2
6	Magnetic stirrer with heating, stainless steel, digital, 280 °C, 100-1500 rpm	FHO-RSM10HS	1
7	Magnetic stirring bar, 50 mm, cylindrical	46299-03	1
8	Erlenmeyer flask, borosilicate, narrow neck, 250 ml	46142-00	1
9	Rubber stopper 26/32, 1 hole 7 mm	39258-01	1
10	Glass tube, straight, l=80 mm, 10/pkg.	MAU-16074541	1
11	PVC tubing, inner dia. = 7 mm, l = 1 m	03985-00	1
12	Graduated cylinder, Borosilicate, 100 ml	36629-00	2
13	Mortar w. pestle, 70ml, porcelain	32603-00	1
14	Sieve, fine mesh, d=60 mm	40968-00	1
15	Graduated pipette, 1 ml	36595-00	1
16	Graduated pipette 10 ml	36600-00	2
17	Beaker, Borosilicate, tall form, 250 ml	46027-00	2
18	Test tubes, 160x16mm, 10pcs	36301-03	1
19	Glycerol 99% 100 ml	30084-10	1
20	Wash bottle, plastic, 500 ml	33931-00	1
21	Hydrogen peroxide, 30%, 250 ml	31710-25	1
22	Hydrochloric acid, 1.0 mol/l, 1000 ml	48454-70	1
23	Caustic soda solution, 1.0 m, 1000 ml	48329-70	1
24	measureAPP - the free measurement software for all devices and operating systems	14581-61	1

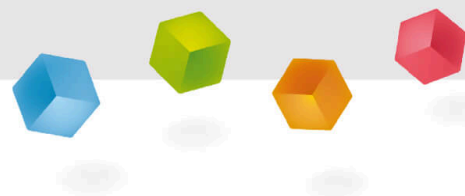
## Additional material

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Position	Art. No.	Designation
1		Mobile device (smartphone / tablet)
2	14581-61	measureAPP
3		Ice cube
4		Kettle
5		Distilled water
6		Small piece of chicken or pork liver

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## Set-up & Procedure





## Set-up (1/3)

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For measurement with the **Cobra SMARTsense sensors** the **PHYWE measureAPP** is required. The app can be downloaded free of charge from the relevant app store (see below for QR codes). Before starting the app, please check that on your device (smartphone, tablet, desktop PC) **Bluetooth** is **activated**.



iOS



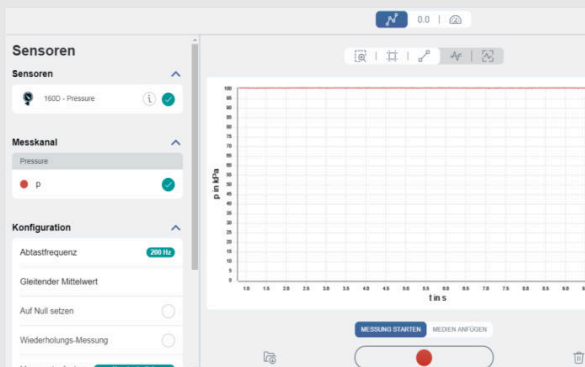
Android



Windows

## Set-up (2/3)

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User interface measureApp  
in the Windows 10 version

- Switch on the SMARTsense Absolute Pressure Sensor by pressing and holding the power button.
- Connect the sensor in the measureAPP under the item "Measure" to the device as shown in the figure on the left.
- The SMARTSense Absolute Pressure Sensor is now displayed in the app.

## Set-up (3/3)

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### Experiment preparation

- Set up the units according to the illustration on the right.
- Place the Erlenmeyer flask on the magnetic stirrer and lock it with the universal clamp and the double sleeve below the pressure module. Screw the glass tube with a little glycerine into the rubber stopper. Then connect the pressure module to the glass tube with a piece of tubing that is as short as possible.



Experimental setup

## Procedure (1/2)

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Put a small piece of liver (cut into small pieces if necessary) into the mortar and add some distilled water. Crush with a pestle and pour the juice through a sieve into the beaker.

### Experiment 1:

- First prepare a just under 0.5% hydrogen peroxide solution: To do this, prepare a 3% hydrogen peroxide solution (10 ml of 30% H<sub>2</sub>O<sub>2</sub>-solution and 90 ml distilled water). Then put 15 ml of the 3% solution into 100 ml measuring cylinder and fill up to 100 ml with distilled water.
- Pour the solution into the conical flask, add the stirring rod and place on the magnetic stirrer.
- Add 1 ml of liver juice and immediately close the conical flask with the rubber stopper.
- Set a small stirring stage and start the measurement (running time 150 s).

## Procedure (2/2)

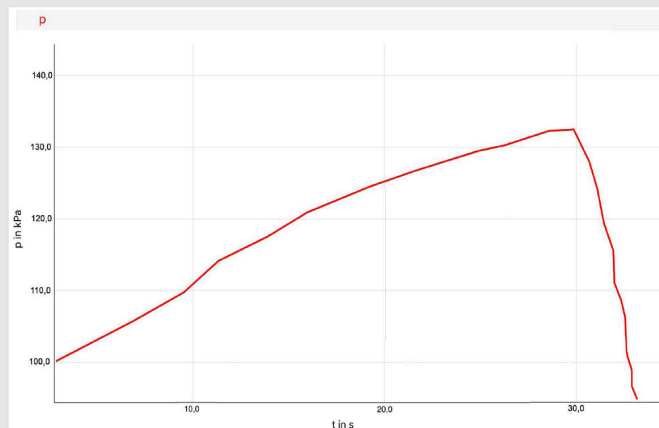
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### Experiment 2a and 2b:

- Carry out as in experiment 1, but still 10 ml **caustic soda** 1 mol/l or 10 ml **hydrochloric acid** Add 1 mol/l.

### Experiment 3a and 3b:

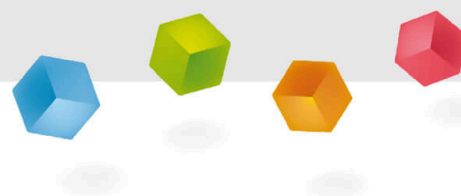
- Carry out as in experiment 1, but put the liver juice into a test tube beforehand and pour it into a beaker with ice-cold (**ice cube**) resp. **boiling water** pose.



Where do you expect this pressure curve?

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## Report



## Task 1

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Select the correct answers.

- ☐ When hydrochloric acid is added, a very clear increase in the pressure curve can be seen. The stopper is pressed out of the Erlenmeyer flask after some time.
- ☐ When hydrochloric acid is added, no increase in the pressure curve can be seen.
- ☐ When sodium hydroxide is added, a smaller increase in the pressure curve can be seen compared to normal conditions.

✓ Check

## Task 2

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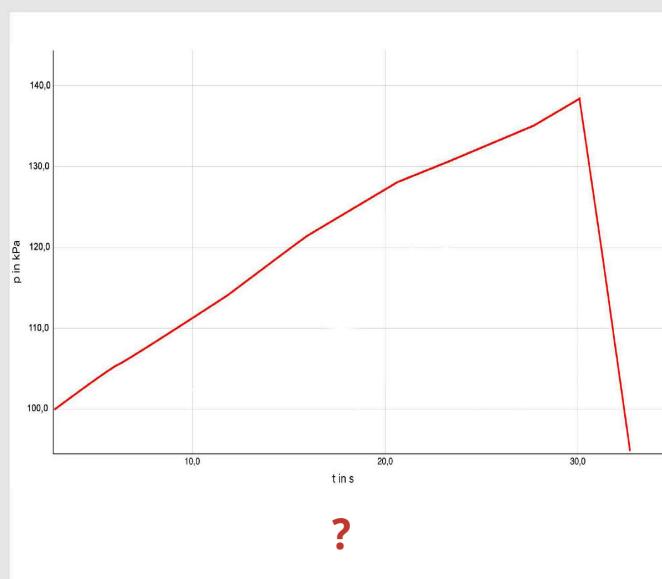
The pressure curve for which experiment is shown on the right?

Experiment 3b: Heat

Experiment 3a: Cold

Experiment 2a: Lye

Experiment 2b: Acid



## Task 3

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Select the correct statements.

- ☐ Catalase is an enzyme that in humans is found mainly in the liver and in erythrocytes.
- ☐ Catalase is an enzyme that in humans is found mainly in the kidney and in white blood cells.
- ☐ Catalase breaks down hydrogen peroxide to water and oxygen.
- ☐ Catalase forms hydrogen peroxide from the water and oxygen present.

✓ Check

Slide	Score / Total
Slide 19: Catalase	0/2
Slide 20: Pressure curve	0/1
Slide 21: Catalase	0/2

Total  0/5

 Solutions

 Repeat