Catalase Activity Assay

The protocol is based on the following reactions:

$$H_2O_2 \xrightarrow{\text{Catalase}} 1/2O_2 + H_2O$$
$$H_2O_2 + 2ABTS\ (reduced) \xrightarrow{HRP} 2ABTS(oxidized) + 1/2O_2 + H_2O$$

The reaction will use a comparison in order to detect differences in 416 nm light absorbance. The amount of catalase oxidase activity will be calculated based on the detected different in absorbance. In the first reaction catalase will decompose hydrogen peroxide. Afterwards, the first reaction will be stopped, and the hydrogen peroxide left will be used as substrate in the second reaction to oxidize ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)). ABTS is an organic material soluble in water, which its oxidized form absorbs 416 nm (green) light. The ABTS will be oxidized by the **horseradish peroxidase** enzyme (HRP), which oxidizes its substrates in the presence of hydrogen peroxide (in a 1:2 ratio). Therefore, the difference in absorbance of 416 nm light indicates us the amount of hydrogen peroxide left, from which we can deduce the activity of catalase.

Materials:

- 23 mL of 0.1 M PB buffer
- 1mL of Horseradish peroxidase (HRP) 330 $\frac{U}{ml}$ solution
  Please prepare this solution close to experiment
  Weigh $1mL * 3\frac{mg}{ml} = 1\ mg$ mg of HRP enzyme to solution and add 1 mL of DW water to tube
- DW
- 1mL of ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) 7.28 mM solution
  Weigh 3.7 mg of ABTS in Eppendorf tube and add 1mL of 0.1M distilled water.
- 1mL of 2M HCl solution.
- Stop-watch
- 1 mL of commercial Catalase (Enzyme) solution, containing 20 U/mL, in different pH levels of 4.5, 5, 6, 7
  Add 980µL of phosphate buffer pH=7.4 to tube. Add 20 µL of catalase solution, containing 1000U/ml to tube.
- For each pH level: 65 mM hydrogen peroxide solution in volume of 6 mL.
  Add 102 µL of Hydrogen peroxide to tube (if you have 30% solution, if not, multiply the amount by 30/(your w/v percentage). Complete volume to 20 mL.
  Please work in a chemical hood!

Procedure:

Notes:

- Please perform all steps in a chemical hood.
- Please perform all steps in triplicate.

1. For each tube, add (in µL):

Reagent	Blank	Supernatant	Lysate	Capsule liquid	1 U/ml	2 U/ml	5U/ml	10 U/ml	15 U/ml	20 U/ml
Substrate (hydrogen peroxide) solution, 65mM	900	900	900	900	995	990	975	950	925	900
Catalase solution	-	-	-	-	5	10	25	50	75	100
Capsule liquid	-	-	-	100	-	-	-	-	-	-
Lysate Solution	-	-	100	-	-	-	-	-	-	-
Supernatant Solution	-	100	-	-	-	-	-	-	-	-
Buffer solution	100	-	-	-	-	-	-	-	-	-

2. Wait precisely 5 minutes, and then add 30 µL of 2M HCl solution. **Please work in a chemical hood!**
3. Add to new tubes:
    - 30 µL of ABTS solution
    - 10 µL of HRP solution
    - 910 µL of 7.4 phosphate Buffer (instructions can be found in the dedicated protocol)
4. Add to each tube 20 µL of Catalase-Hydrogen peroxide solutions
5. Wait precisely 5 minutes, and then add 30 µL of 2M HCL.
   Please work in a chemical hood!
6. Add 200 µL of each tube and read absorbance in 416nm light.
7. For each pH level $\Delta A_{416nm} = A_{Blank} - A_{Sample}$