Luciferase

In an experiment, mitochondria were isolated and placed in a solution at pH 7. Samples from these isolated mitochondria were then incubated under different conditions as described in the table below. ATP production was measured using the enzyme luciferase, which catalyzes the formation of the following reaction:

Luciferan + ATP +
$$O_2 \rightarrow Oxyluciferin + AMP + PPi + CO_2 + Light$$

The amount of light released (in RLU) is used as an indirect measure of ATP production.

The following data were obtained:

Tube number	Reaction mixture	Reaction mixture pH	Luciferase activity (RLU±sd) Raw data
1	Mitochondria + ADP + Pi + Mg ions (Mg ²⁺) + O ₂	7	12±3
2	Mitochondria + ADP + Pi + Mg ions (Mg ²⁺) + O ₂	3.5	156±17
3	Mitochondria + ADP + Pi + O ₂	3.5	36±5
4	ADP + Pi + Mg ions (Mg ²⁺) + O ₂	3.5	7±2

- a- What is the purpose of this study? To determine optimal conditions for ATP production by oxidative phosphorylation in mitochondria using an indirect method to measure ATP production (through the reaction of luciferase)
- b- Which reaction mixture(s) is(are) the positive control (*give tube number*)? Negative control? Positive control: reaction 2; negative control: reaction 4
- c- Why was O₂ added to the reaction mixtures? Since ATP production is dependent on the presence of)2 in mitochondria (cellular respiration) where it acts as the final electron acceptor in the electron transport chain.
- d- Why did ATP production go up at pH 3.5? The proton motive force is higher at lower pH
- e- What is the role of Mg²⁺ in ATP formation? It acts as a cofactor for enzymes involved in redox reactions in the electron transport chain.
- f- The raw data need to be corrected by taking into consideration the basal level of ATP production (that occurs regardless of the treatment). Results of which reaction mixture would you use to correct/adjust the data? justify your answer. The basal level of ATP production is depicted in reaction 4 (absence of mitochondria). This basal level can be subtracted from the values obtained in the other reaction mixtures.
- g- Plot a graph that shows in a meaningful way ATP production under the conditions tested in this experiment. Label your graph appropriately. Bar graph
- h- Which statistical test would you use in order to analyze the data presented above? Single factor ANOVA.