Hons practical MC 4

GROUPS:

1A
- **Carol Nekatambe**
- **Greta De Waal**

2B
- **Melinda Badenhorst**
- **Yasmin Masoudi**

3C
- **Nicole Green**
- **Tammy Speelman**

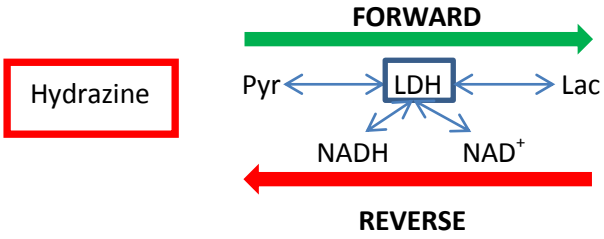
4D
- **Stefan Botha**
- **Will de Villiers**

5A
- **Kay Leukes**
- **SinJon Heitmann**

6B
- **Ané Louw**
- **Lauren Myburg**
- **Anna-Mart Burger**

ABOUT THE PRAC:

(1) Characterise the enzyme:



(2) The experiments:

GROUP A
- Reverse direction
- Varying [NAD^+]
- Same [Lac] +[LDH] + [Hydrazine]

GROUP B
- Reverse direction
- Varying [Lac]
- Same [NAD^+] +[LDH] + [Hydrazine]

GROUP C
- Forward direction
- Varying [NADH]
- Same [Pyr] +[LDH]

GROUP D
- Forward direction
- Varying [Pyr]
- Same [NADH] +[LDH]

GROUP A

- Product Inhibition: NADH
- Varying [NADH]
- Same [Lac] +[NAD^+] + [LDH] + [Hydrazine]

GROUP B

- Without Hydrazine
- Varying [Lac]
- Same [NAD^+] +[LDH]

GROUP C

- Product Inhibition: NAD^+
- Varying [NAD^+]
- Same [Pyr] +[NADH]+[LDH]

GROUP D

- Product Inhibition: Lac
- Varying [Lac]
- Same [NADH] + [Pyr]+[LDH]

(3) An enzyme assay:

a- Pipette metabolites into the cuvettes, the reaction is initiated by the addition of the enzyme.

b- Once the enzyme is added measure the absorbance readings for the reaction over a period of 3mins.

c- The types of data we expect to see- Forward reaction: decreasing Abs; Reverse reaction: increasing Abs

(4) What needs to be done:

<u>Create a pipetting schema so that we investigate 10 different concentrations around the Km values. Take the following into consideration:</u>

a- Given :

Km	Stock concentrations
Km(NADH) = 0.05 mM	[NADH] = 1.5 mM
Km(NAD^+) = 0.4 mM	[NAD] = 5 mM
Km(Pyr) = 0.5 mM	[Pyr] = 10 mM
Km(Lac) = 10 mM	[Lac] = 1 M

HYDRAZINE is given at a 10X dilution of bottle concentration

b- Volumes:

- The total cuvette volume is 1 ml and of that you use 10 µl of the enzyme (LDH) and if you are doing **reverse direction experiments (only)** 35 µl is the Hydrazine.
- The other volumes are of the substrate and co-factor which you calculated in the pipetting schema.
- Lastly, the remaining volume to make up this 1 ml volume is done by adding buffer to the cocktail mixture.

c- How to determine the 10 concentrations:

- The range you want to investigate is between: [Km/10; Km) and (Km; 10Km] and the fixed substrates are usually at saturating condition ie. 10xKm. Use Excel to create an incremental pipetting schema.

(5) The Program:

18/04 Tuesday:	**19/04 Wednesday:**	**20/04 Thursday:**
Morning: Practical theory and pipetting schema Afternoon: Group 1-3 (first set of experiments)	Morning: Group 4-6 (first set of experiments) Afternoon: Group 1-3 (second set of experiments)	Morning: 09H30 Research lecture Mid morning: Group 4-6 (second set of experiments)

21/04 Friday: Morning: Data pooling/ initial data analysis Afternoon: redo experiments?	**24/04 Monday:** Morning: Data analysis/ Redo experiments?	**25/04 Tuesday:** Data analysis day
26/04 Wednesday: Data analysis day	**27/04 Thursday:** **FREEDOM DAY (PUBLIC HOLIDAY)**	