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## Purification of Lactate Dehydrogenase

Irene J. Wilson  
*Dominican University of California*

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# Purification of Lactate Dehydrogenase



**Irene J. Wilson**

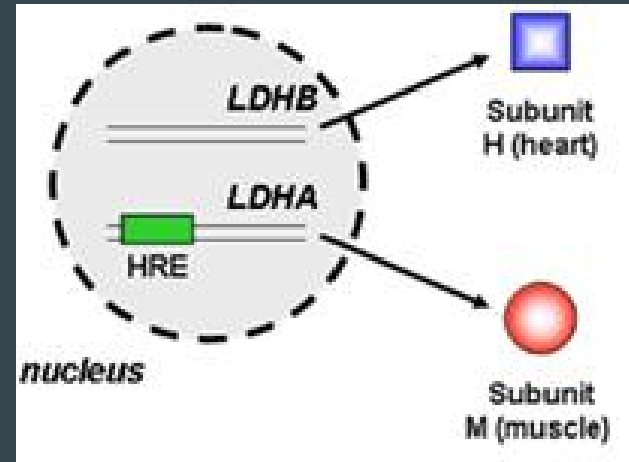
Dominican Scholarly and Creative Works Conference  
Spring 2016

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# Introduction to Lactate Dehydrogenase (LDH)

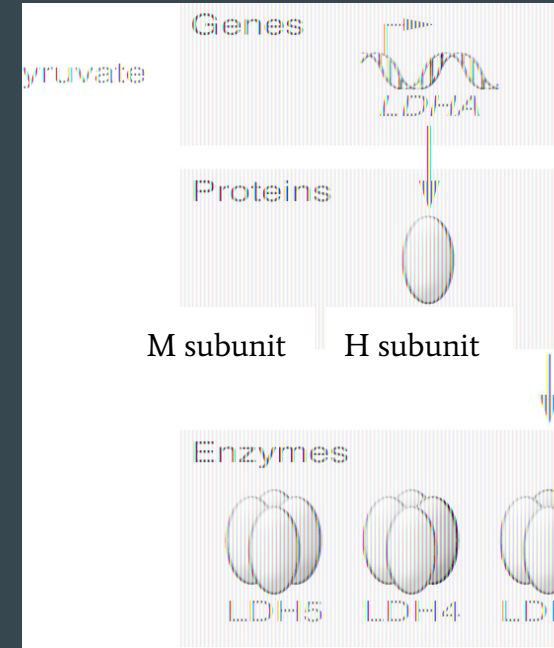
- 3 genes provide instructions for building LDH (protein subunits of LDH):
  - *lactate dehydrogenase A* (LDHA) = M subunit
  - *lactate dehydrogenase B* (LDHB) = H subunit
  - *lactate dehydrogenase C* (LDHC)



# Introduction to Lactate Dehydrogenase (LDH)

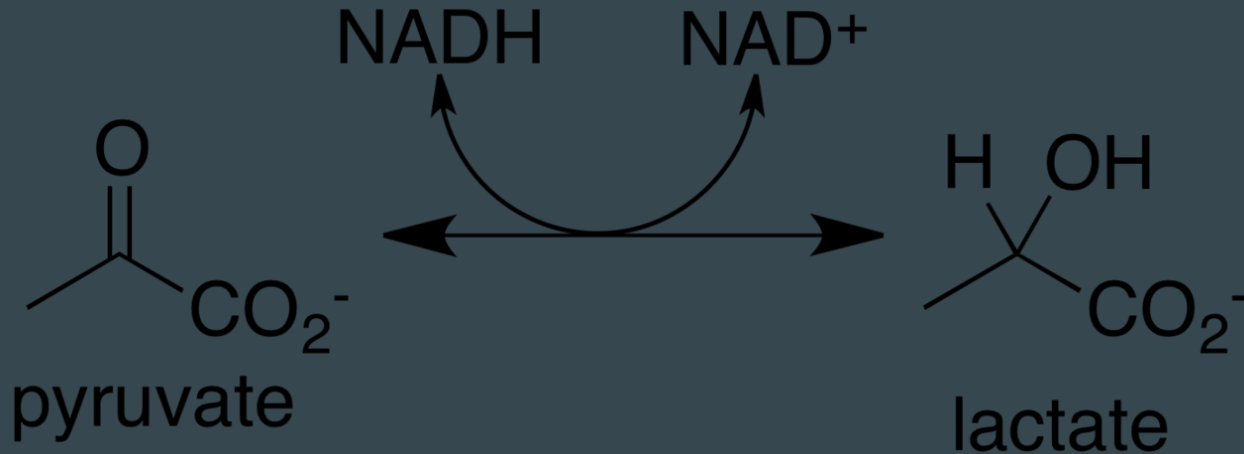


- Tetramer- composed of a combination of four subunits
  - Molecular weight of tetramer is about 140 kDa
- Tetramer made of 2 subunit types: **M** and **H**
  - Molecular weight of each subunit is about 35 kDa
- Five possible isoforms exist:
  - LDH 1 - ( $H_4$ )
  - LDH 2 - ( $H_3M$ )
  - LDH 3 - ( $H_2M_2$ )
  - LDH 4 - ( $HM_3$ )
  - LDH 5 - ( $M_4$ )



# Introduction to Lactate Dehydrogenase (LDH)

- Enzyme found in the cytoplasm of almost all living cells
- All isoforms catalyzes this reversible reaction:

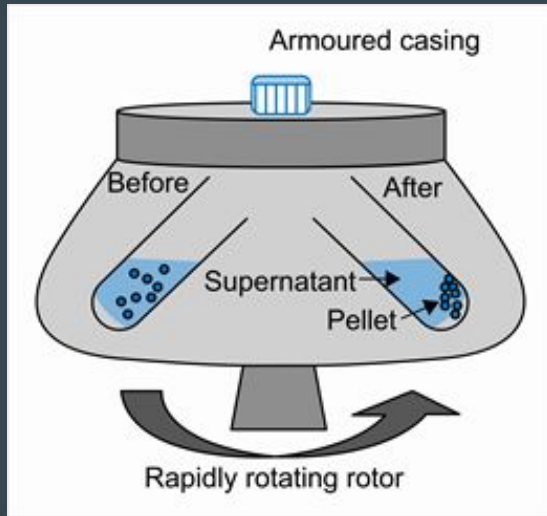


# Summary of Materials and Methods

- Goal of the project was to purify LDH from a cow heart
- Techniques used to purify LDH:
  - Homogenization
  - Centrifugation
  - Ammonium Sulfate Precipitation
  - Gel-filtration Chromatography
- Colorimetric Assay to quantify total protein yields
- Techniques used to verify presence of LDH:
  - LDH Enzyme Activity Assay
  - Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)
  - Coomassie-stained Gel
  - Western Blot with anti-LDH antibody

# Purification of LDH

- Homogenization, centrifugation, and ammonium sulfate precipitation
  - Creation of the pellet (B5), which is enriched in LDH

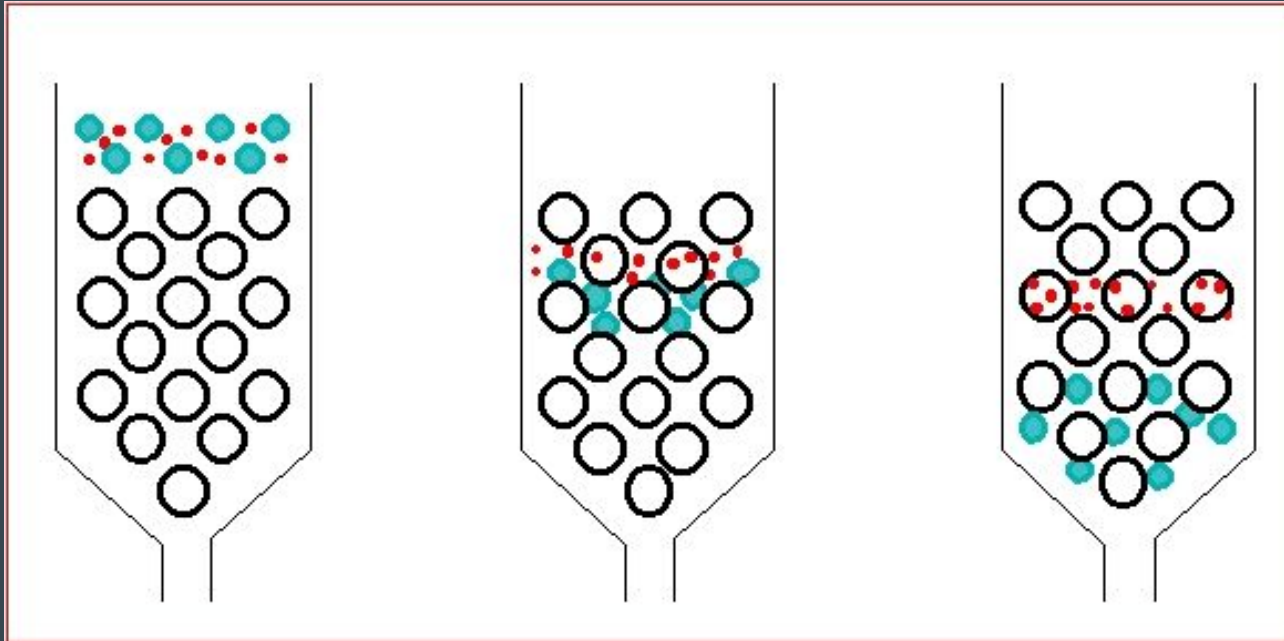


<b>Total volume of beef heart:</b>	35.0 mL
<b>Volume of supernatant in 1st conical tube:</b>	27.5 mL
<b>Amount of ammonium sulfate powder for 40% cut:</b>	6.10 g
<b>Volume of supernatant in 2nd conical tube:</b>	28.0 mL
<b>Amount of ammonium sulfate powder for 65% cut:</b>	4.48 g
<b>Volume of supernatant in 3rd conical tube:</b>	28.0 mL
<b>Volume of resuspended pellet:</b>	1.5 mL



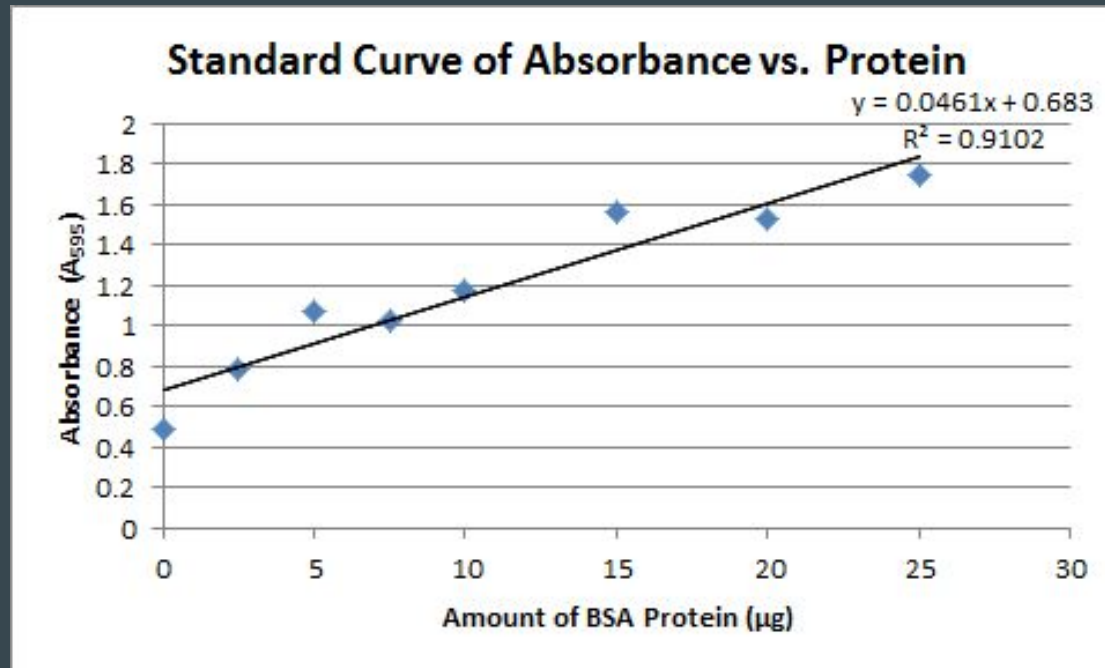
# Purification of LDH

- Gel Filtration Chromatography - separate proteins by size
  - 16 fractions of the sample were collected



# Colorimetric Assay to Quantify Total Protein Yields

- Measured absorbance of varying volumes of a BSA protein standard (1  $\mu\text{g}/\mu\text{L}$ )
- Obtained best-fit line to determine the amount of protein in column fractions



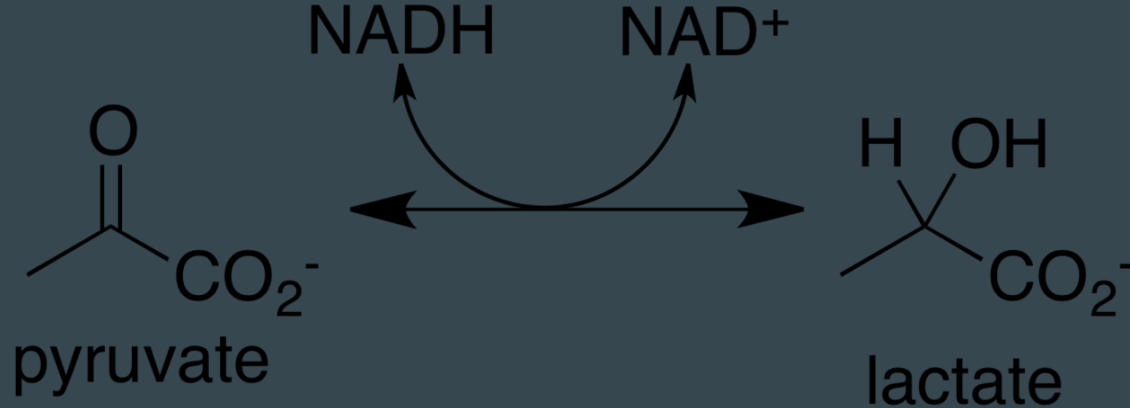
# Colorimetric Assay to Quantify Protein Yields

- Used BSA standard curve to calculate the total protein concentration of the protein-containing fractions
- F1-F12 column fractions and B1-B3 and B5 centrifugation fractions contained protein

Fraction #	Sample Volume (μL)	Average (A)	Protein (μg)	Protein (μg/μL)
F1	7	1.15	10.1	1.44
F2	7	1.54	18.6	2.66
F3	7	1.55	18.8	2.69
F4	7	1.72	22.5	3.21
F5	7	1.23	11.9	1.7
F6	7	1.42	15.9	2.27
F7	7	1.22	11.6	1.66
F8	7	0.936	5.49	0.784
F9	7	0.944	5.66	0.809
F10	7	0.75	1.45	0.207
F11	7	0.709	0.564	0.0806
F12	7	0.733	1.08	0.154
F13	7	0.63	0	0
F14	7	0.616	0	0
F15	7	0.606	0	0
F16	7	0.551	0	0
B1	2	2.032	29.3	14.65
B2	2	1.74	22.9	11.45
B3	2	1.32	13.8	6.9
B4	2	0.575	0	0
B5	2	1.77	23.6	11.8

# Verification of LDH

- LDH Enzyme Activity Assay
- Measures the rate at which  $\text{NAD}^+$  and lactate are converted to pyruvate and NADH
- Production of NADH monitored as increase in absorbance at 340 nm



# Verification of LDH

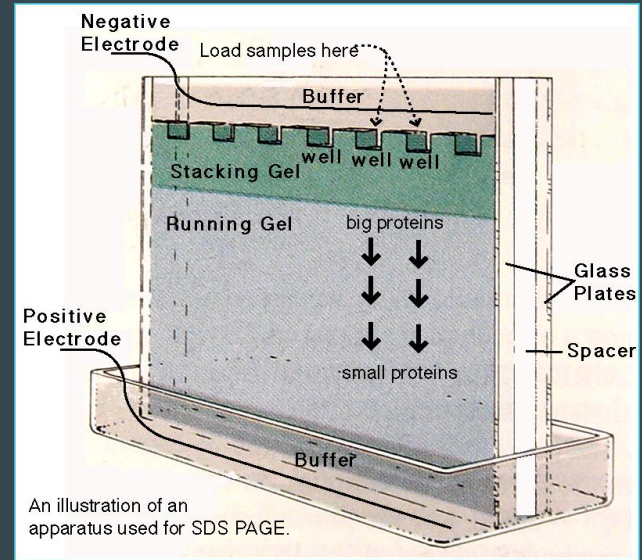
- Assayed all protein-containing fractions
- F2-F6 column fractions and B1-B5 centrifugation fractions contained LDH
- Used the 2 column fractions with the highest rate of measured LDH activity (F3 and F4) and the centrifugation fraction B5 (pellet) for visualization by SDS-PAGE

<b>Fraction</b>	<b><math>\Delta</math> Abs/ <math>\Delta</math> min.</b>	<b>Relative Activity (Units/mL)</b>
F1	0	0
F2	0.002	0.096
F3	0.093	0.748
F4	0.053	2.556
F5	0.045	2.170
F6	0.016	0.772
F7	0	0
F8	0	0
F9	0	0
F10	0	0
F11	0	0
F12	0	0
B1-Crude	0.107	10.322
B2-Sup1	0.153	7.379
B3-40 Sup	0.151	7.283
B4-65 Sup	0.027	1.860
B5-65 Pellet	0.244	16.812

# SDS-PAGE

## (Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis)

- SDS eliminates differences in protein shape and native charge
  - All proteins migrate based on chain length/molecular weight
- Gel was cut in half:
  - One half stained with Coomassie Blue stain to detect presence of total proteins
  - One half transferred to a membrane via Western blotting





# Coomassie Blue Stain of SDS-PAGE Gel

Lanes:

1

2

3

4

5

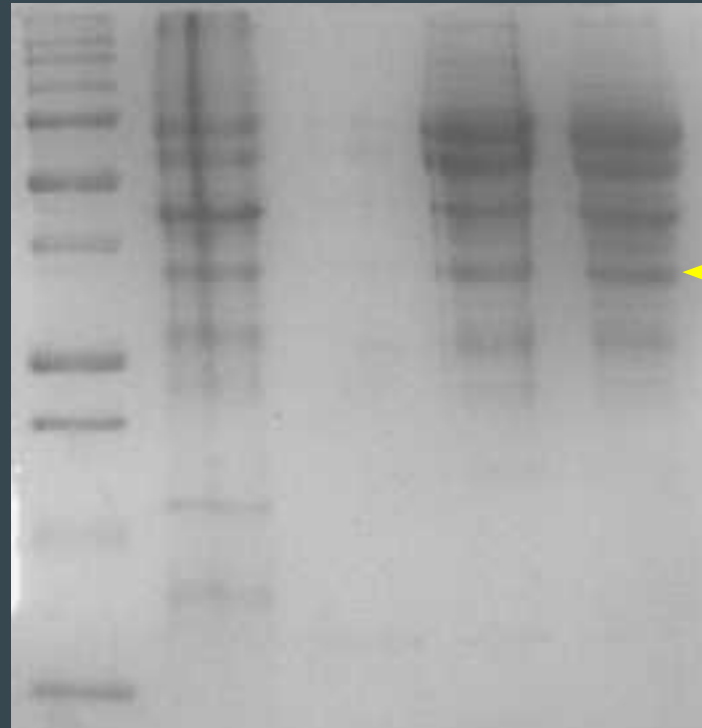
Protein  
Marker

B5  
Pellet

LDH  
Standard

F3  
Sample

F4  
Sample

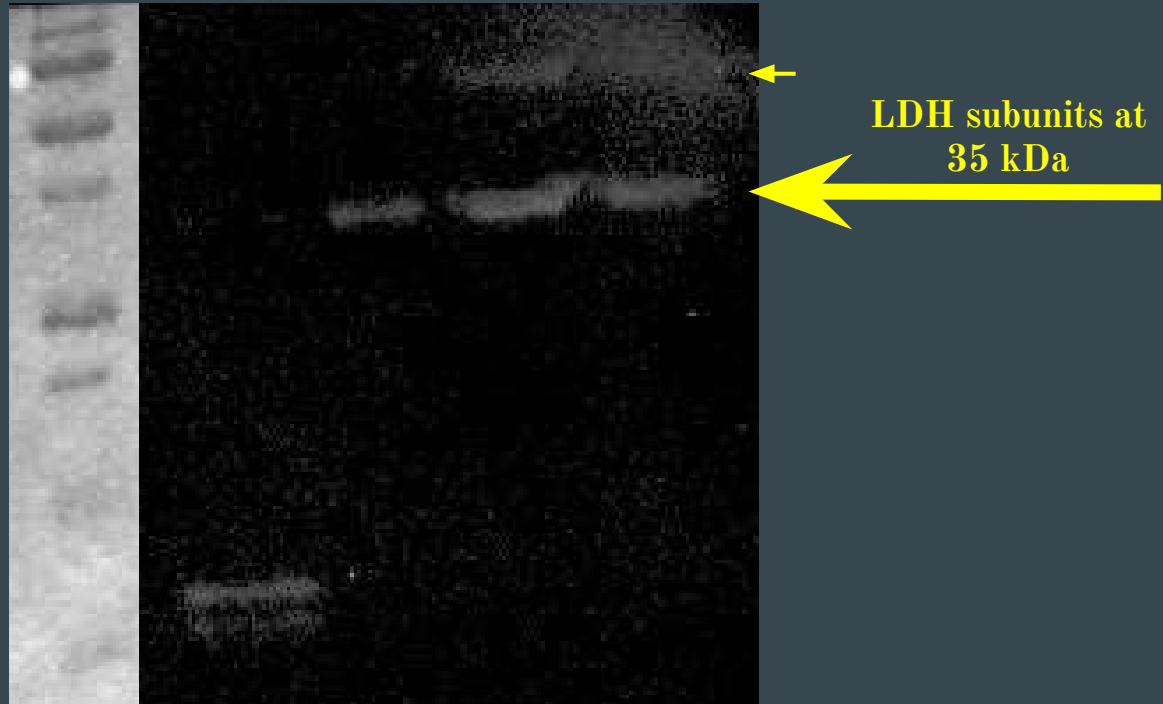


LDH subunits at  
35 kDa



# Western Blot developed with anti-LDH antibody

Lanes:    1            2            3            4            5  
              Protein    LDH        B5        F3        F4  
              Marker    Standard   Pellet    Sample   Sample



# Conclusion

- Goal of the project was to purify LDH from a cow heart
- LDH was successfully purified from a cow heart
  - Samples F3-F5 contained LDH
  - Purification can be improved
- Ideal for undergraduate academic setting:
  - Learn valuable laboratory techniques
  - Collect, analyze, and critique results
  - Practice responsibility and critical thinking

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My parents