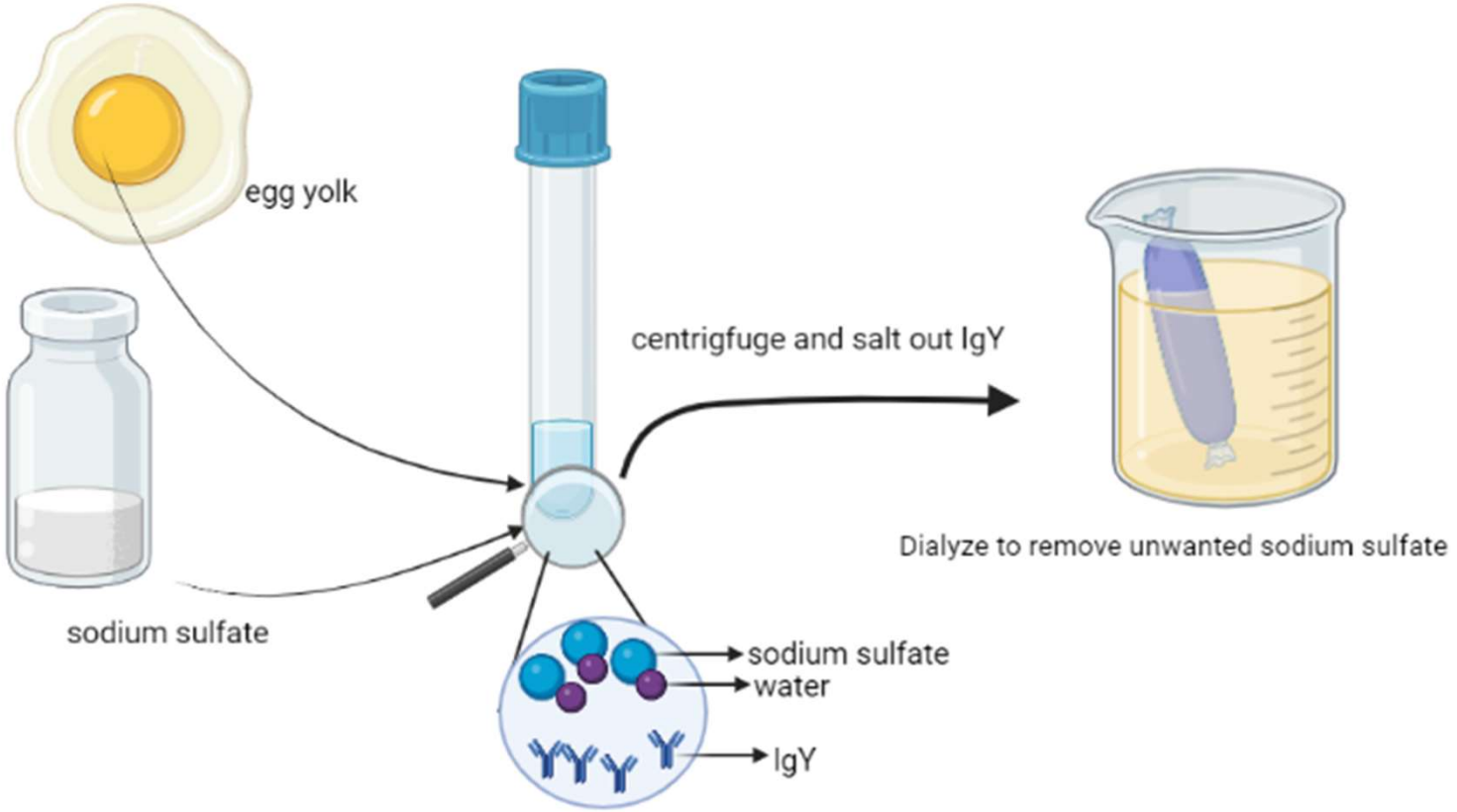
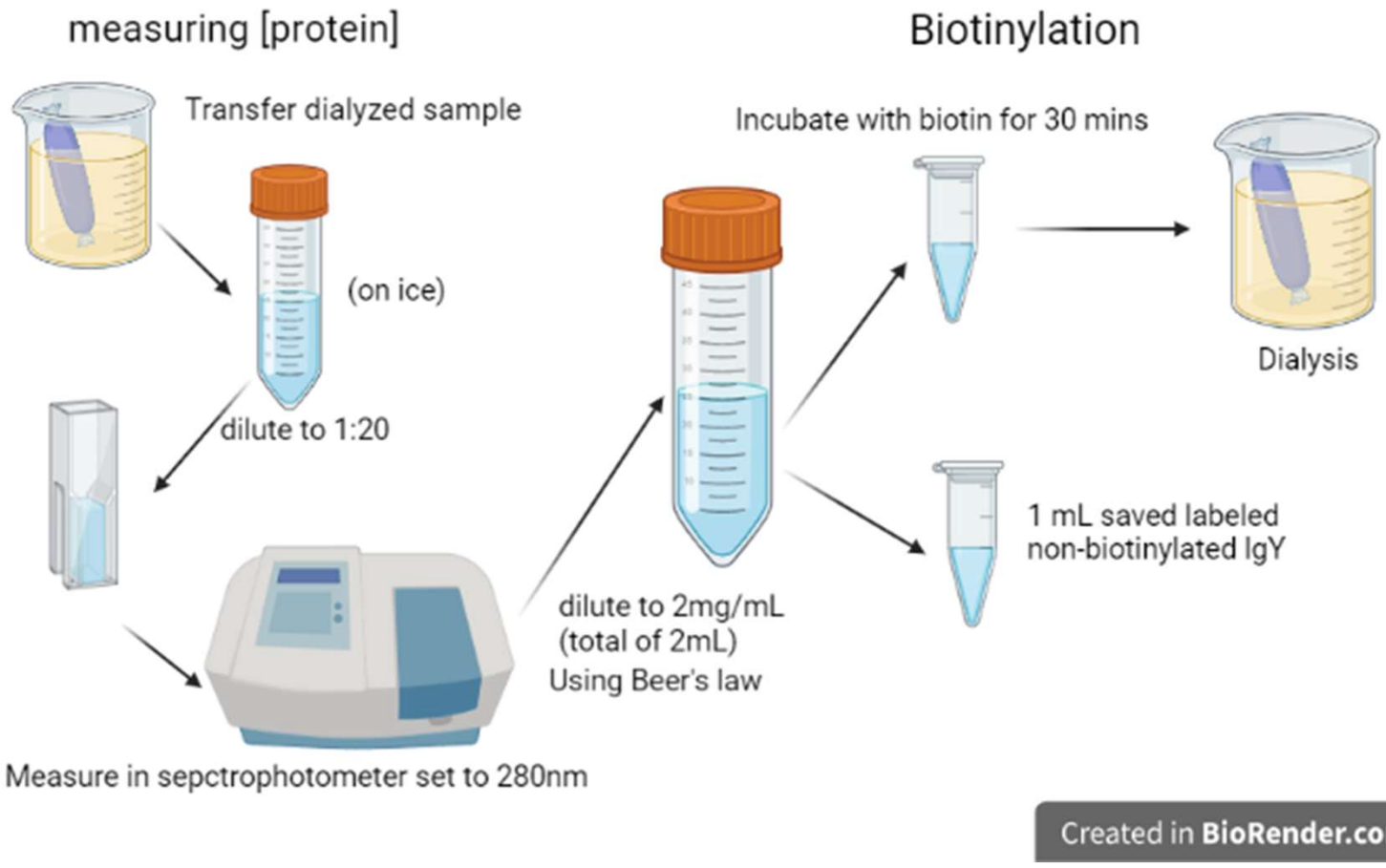
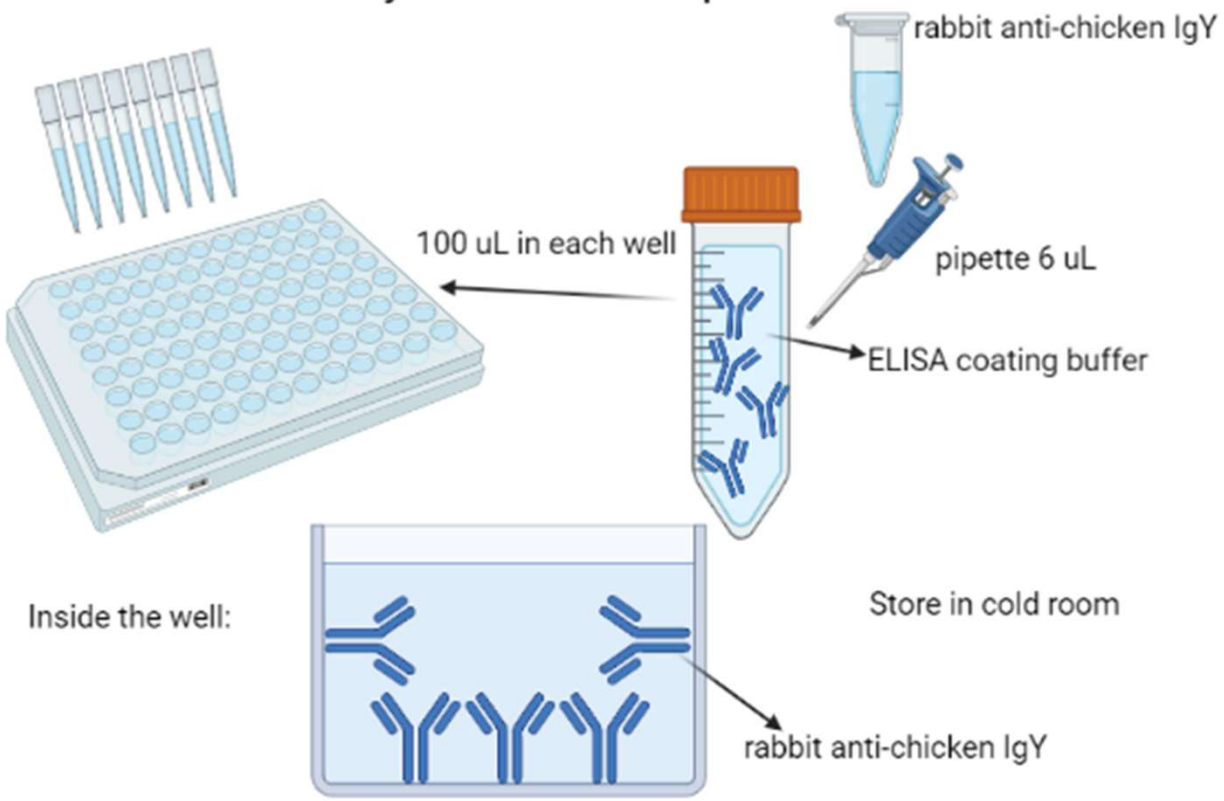
Day 1: Isolation of IgY



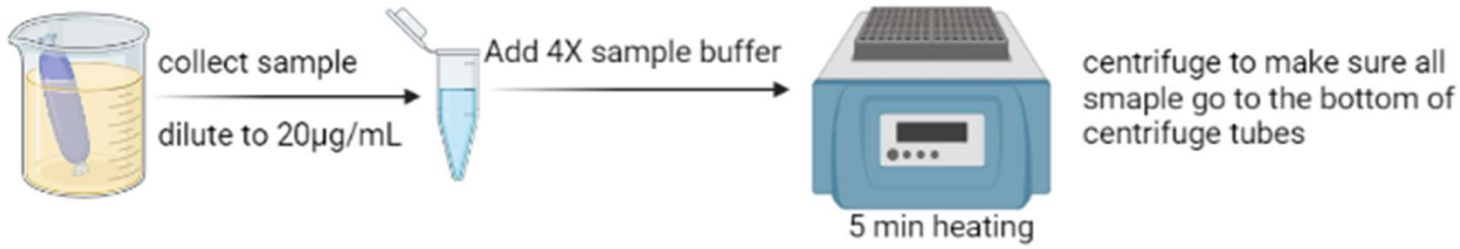
Day 2: Biotinylation of IgY

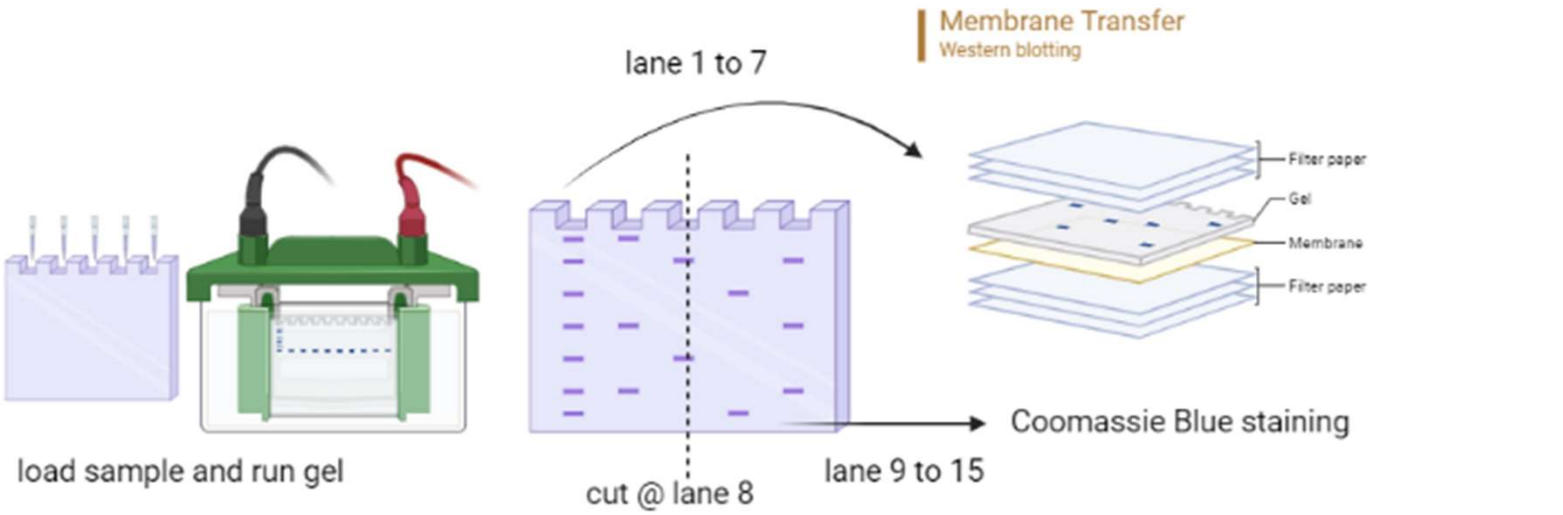


Day 2: ELISA setup



Day 3: SDS-PAGE and Western Blot





Day 3: ELISA (continued)

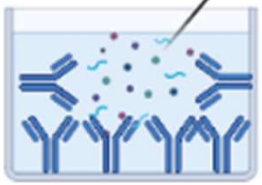
ELISA plate from day 2

3 X wash with:

PBST

100 uL in each well

1X PBS +1% bovine
(blocking solution)



antibodies attached on
the wall of wells

200 uL in each well

flick well and tap out liquid

Excess unwanted molecules
removed
without damaging antibodies

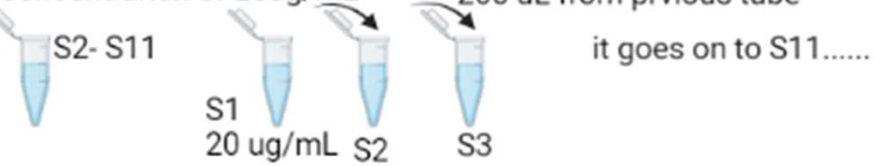
well

the BSA block the surface of the well to prevent biotinylated IgY from attaching the inner surface of well instead of binding to the antibodies.

Day 4: Elisa Continued

S1-S12: diluted with sample concentraiton of 20ug/mL

300 uL PBST +1%BSA

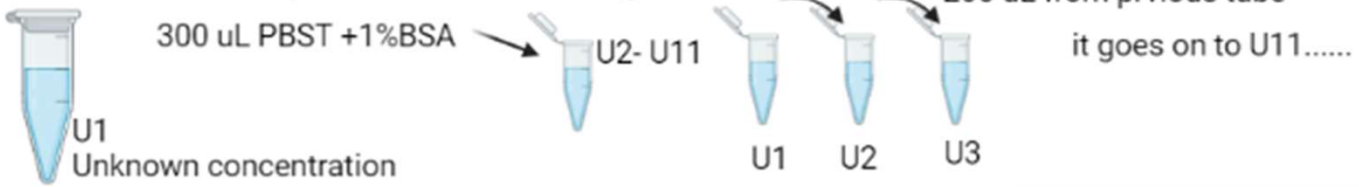


200 uL from prvious tube

it goes on to S11.....

U1-U12: diluted with sample concentraiton of 20ug/mL

300 uL PBST +1%BSA

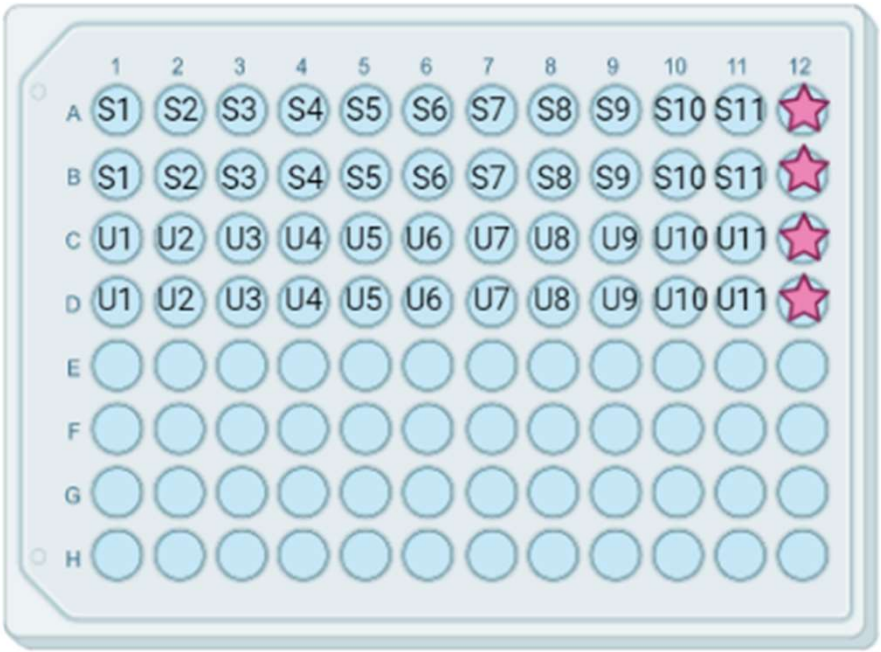


200 uL from prvious tube

it goes on to U11.....

Day 4: ELISA Continued

Pipette prepated diluted samples into wells as the graph below



100 uL PBST +1% BSA

Day 5: Finishing ELISA

After 3x times PBST washes:

100 µL of PBST + Streptavidin-AP to each well

Incubate for 1 hour

Tap out liquid, do another 3x times PBST washes

Add substrate solution to each well and start timing

Record absorbance at 405 nm at 0, 10, 20, 30 mins

color trend should look like this

using a plate reader

Inside each well: