

Magnetic Bead Purification and ELISA

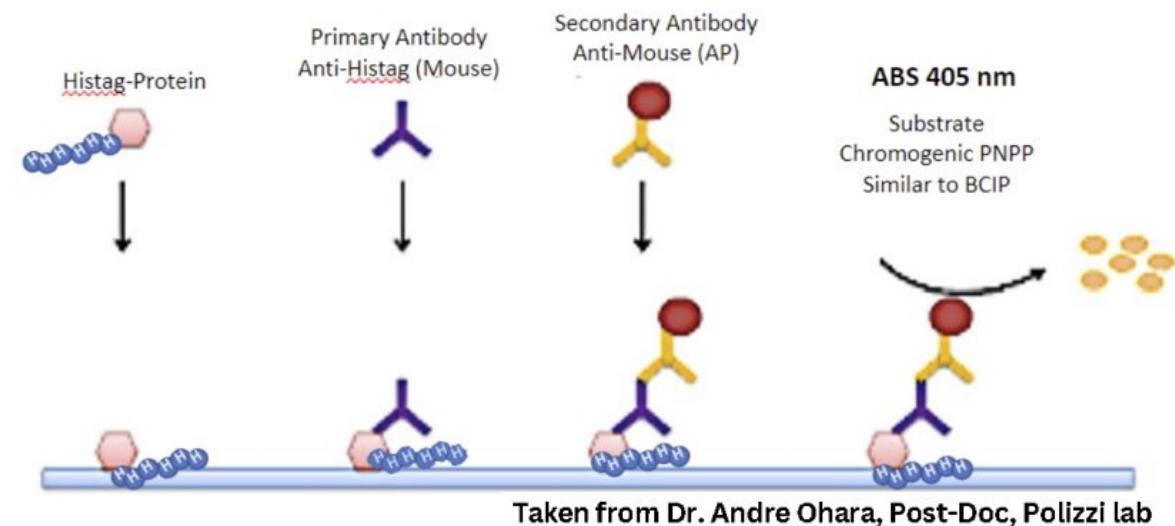
Team 4

Chelsea Dack, Jonathan Foldi, Jeremy Chua, Anwesha Mohapatra, Ayushi Katdare, Kalyan Ghadiyaram

Indirect ELISA



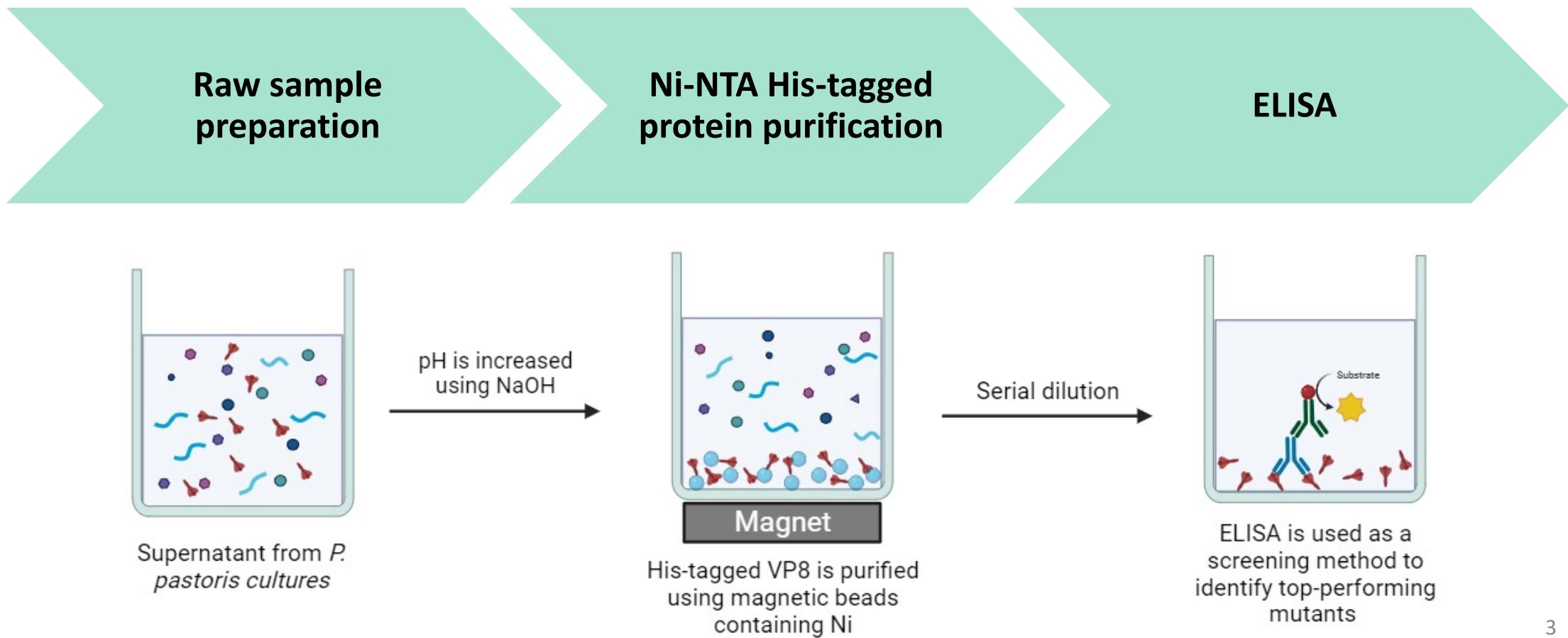
- ELISA (Enzyme-Linked ImmunoSorbent Assay) quantitative method for proteins, antibodies, antigens, etc in biological samples
- Protein > 1^o antibody > 2^o antibody conjugated to enzyme > substrate > emits coloured signal
- Manually intensive – lots liquid handling & incubation steps
- Automation would improve efficiency, reduce variability, and increase throughput



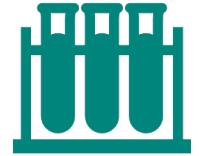
Polizzi group's protocol



Screening *P. pastoris* mutants for a His-tagged VP8 protein:



Planning – special equipment



- Custom labware:
 - ThermoFisher ELISA 96 well plate (400 µL)
- Magnetic module:
 - Flat bottom adapter

Custom Labware Creator BETA

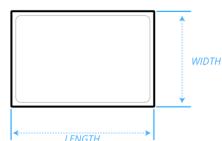
[READ THE CUSTOM LABWARE GUIDE](#)



Total Footprint

Ensure measurement is taken from the very bottom of labware.

The footprint measurement helps determine if the labware (in adapter if needed) fits firmly into the slots on the OT-2 deck.



Length	127.7	mm
Width	85.4	mm

Total Height

Include any well lip in the measurement. Exclude any cover or cap.

The height measurement informs the robot of the top and bottom of your labware.



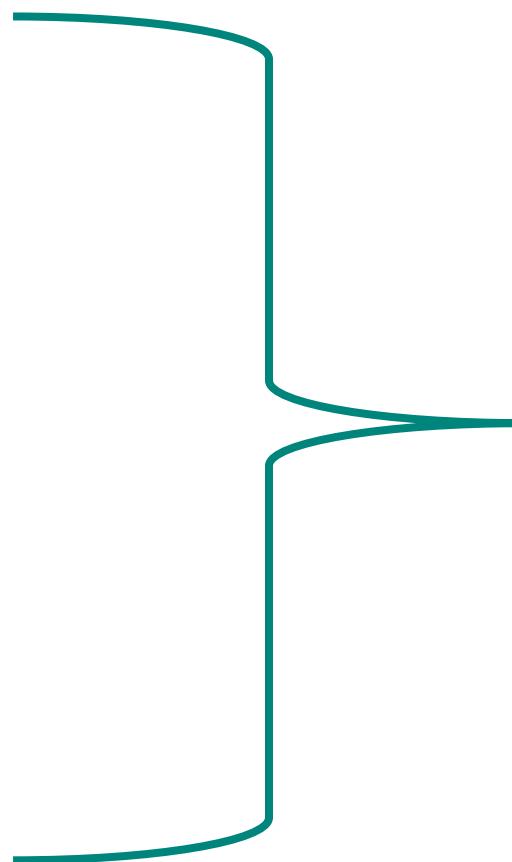
Height	14.4	mm
--------	------	----



Planning – volumes

To accommodate 16 samples, these reagents are required:

- 6.5 mL NaOH
- 20 mL Ni-NTA equilibrium buffer
- 1 mL N-NTA bead slurry
- 20 mL Ni-NTA wash buffer
- 3.5 mL Ni-NTA elution buffer
- 8 mL ELISA coating solution
- 20 mL ELISA blocking buffer
- **87 mL ELISA PBS-T**
- 10 mL ELISA 1^o antibody
- 10 mL ELISA 2^o antibody
- 10 mL ELISA PnPP
- **LOTS of waste**



**Lots of reagents
Lots of liquids
Lots of tips**

W	W	W	W	W	W	W	W	p		p
W	W	W	W	W	W	W	W	P	w	p



Liquid handling

```
def which_PBS(current_PBS,reservoir,trash):
    if current_PBS<20000:
        return(trash["A8"]) ← Well to use
    elif current_PBS<40000:
        return(trash["A9"])
    elif current_PBS<60000:
        return(trash["A10"])
    elif current_PBS<80000:
        return(trash["A11"])
    elif current_PBS<100000:
        return(trash["A12"])
    elif current_PBS<120000:
        return(reservoir["A8"])
    elif current_PBS<140000:
        return(reservoir["A12"])

def run(protocol:protocol_api.ProtocolContext):
    current_PBS = 0 ← Total volume so far
    current_trash = 0

    current_PBS+=800*12 ← Volume for the step
    p300.transfer(100,which_PBS(current_PBS,reservoir,trash) , plate.rows()[0][11::-1]
    current_trash += 800*12
    p300.transfer(100,plate.rows()[0][11::-1], trash[which_trash(current_trash)])
```

```
def which_trash(current_trash):
    if current_trash<22000:
        return("A1")
    elif current_trash<44000:
        return("A2")
    elif current_trash<66000:
        return("A3")
    elif current_trash<88000:
        return("A4")
    elif current_trash<110000:
        return("A5")
    elif current_trash<132000:
        return("A6")
    elif current_trash<154000:
        return("A7")
    elif current_trash<176000:
        return("A8")
    elif current_trash<198000:
        return("A9")
    elif current_trash<215000:
        return("A10")
    elif current_trash<232000:
        return("A11")
```



Liquid handling

```
def which_PBS(current_PBS,reservoir,trash):
    if current_PBS<20000:
        return(trash["A8"])
    elif current_PBS<40000:
        return(trash["A9"])
    elif current_PBS<60000:
        return(trash["A10"])
    elif current_PBS<80000:
        return(trash["A11"])
    elif current_PBS<100000:
        return(trash["A12"])
    elif current_PBS<120000:
        return(reservoir["A8"])
    elif current_PBS<140000:
        return(reservoir["A12"])

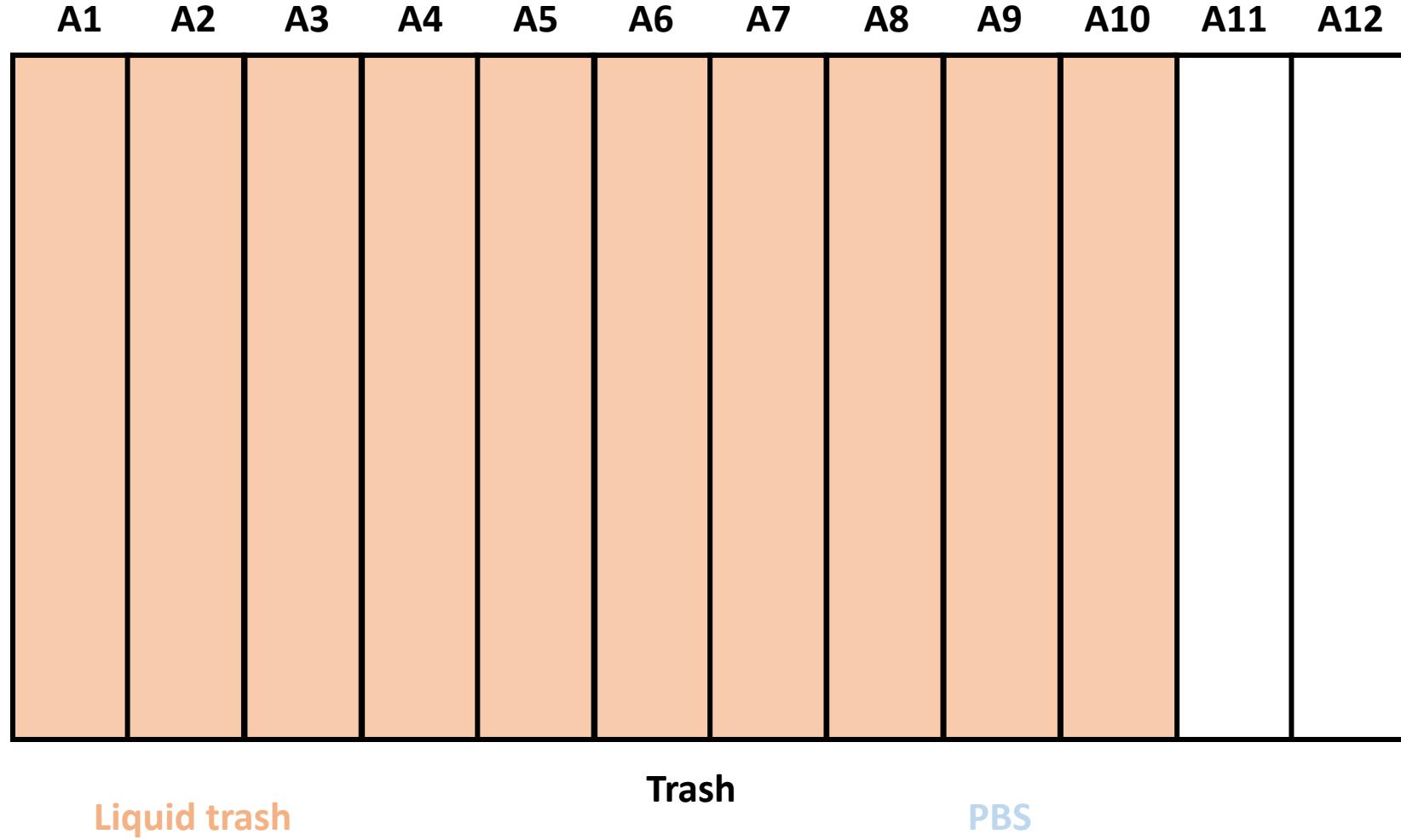
def run(protocol:protocol_api.ProtocolContext):
    current_PBS = 0
    current_trash = 0

    current_PBS+=800*12
    p300.transfer(100,which_PBS(current_PBS,reservoir,trash) , plate.rows()[0][11::-1]
    current_trash += 800*12
    p300.transfer(100,plate.rows()[0][11::-1], trash[which_trash(current_trash)])
```

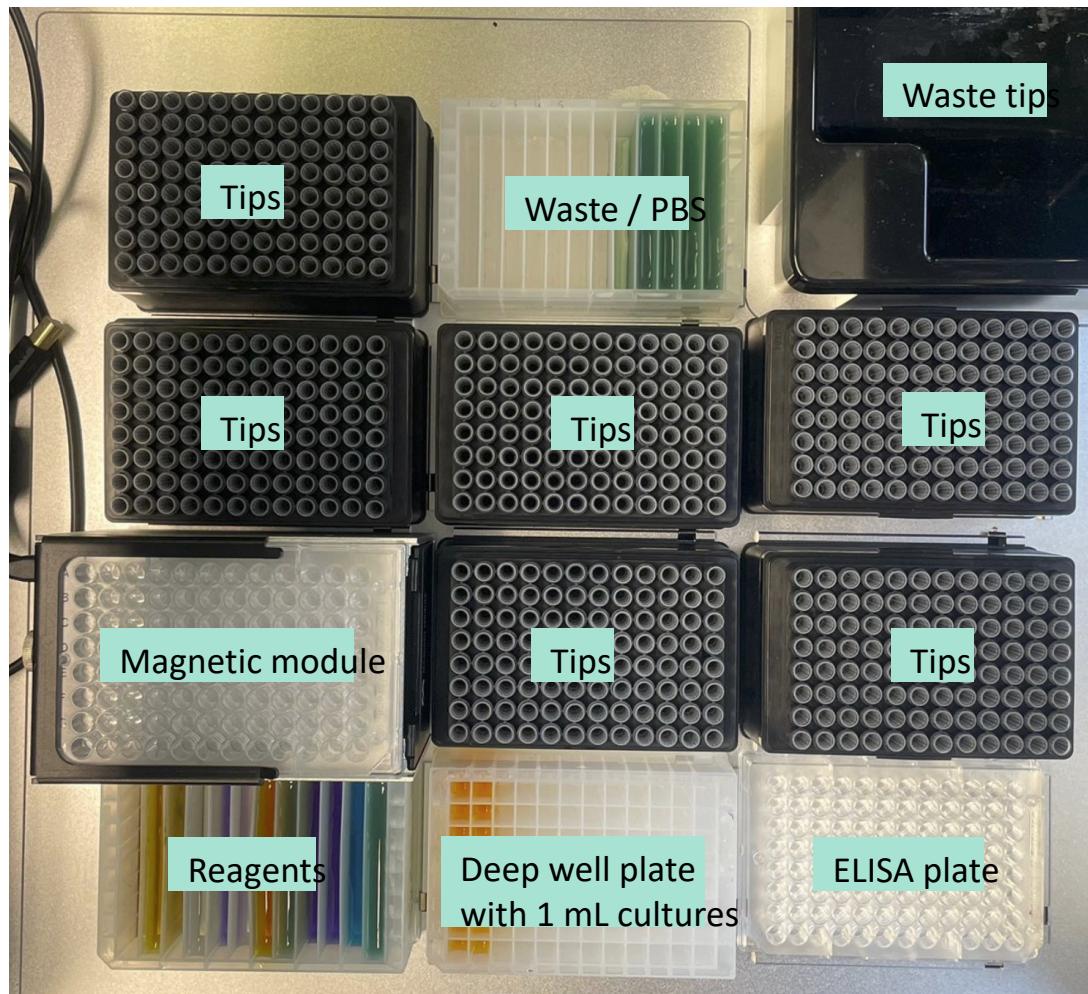
Reused PBS
well for waste

```
def which_trash(current_trash):
    if current_trash<22000:
        return("A1")
    elif current_trash<44000:
        return("A2")
    elif current_trash<66000:
        return("A3")
    elif current_trash<88000:
        return("A4")
    elif current_trash<110000:
        return("A5")
    elif current_trash<132000:
        return("A6")
    elif current_trash<154000:
        return("A7")
    elif current_trash<176000:
        return("A8")
    elif current_trash<198000:
        return("A9")
    elif current_trash<215000:
        return("A10")
    elif current_trash<232000:
        return("A11")
```

Liquid handling – reusing wells

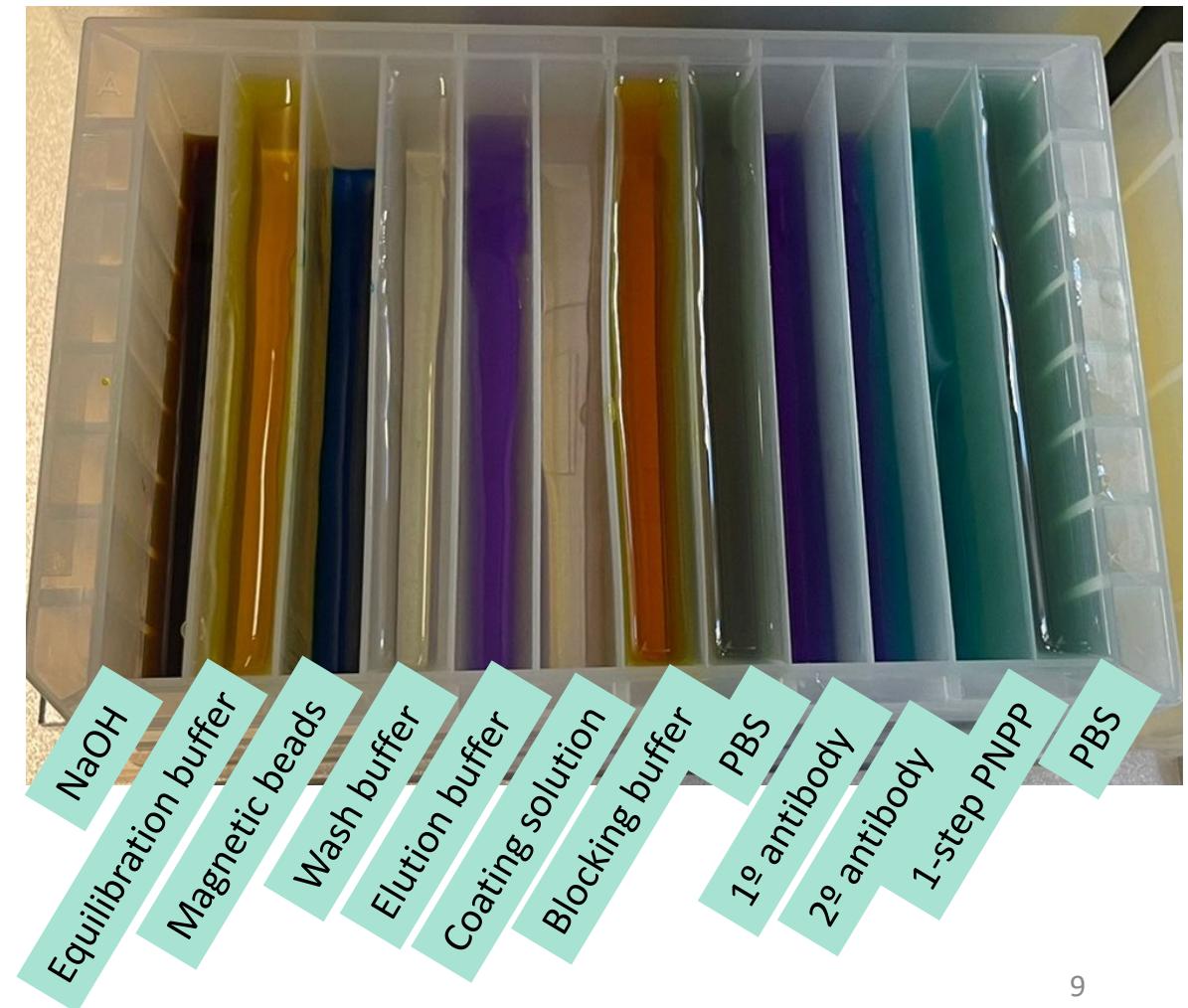


Deck set up



- P300 Multi-channel pipette

Reagents



Sample preparation

Prep

Ni-NTA

ELISA

1 & 2. Extract culture supernatant & increase pH with NaOH

```
#####
# EXTRACT SUPERNATANTS, CORRECT pH, DUPLICATE SAMPLES
#####

# ADD 80 µL NaOH TO SUPERNATANT SAMPLES
p300.transfer(80, reservoir["A1"], deep_well.rows()[0][2:4], blow_out = True), mix_after=(3, 50))

# 1 mL CENTRIFUGED CULTURES IN SLOT 2 DEEP WELL PLATE, COLUMNS 1 & 2
# EXTRACT 800 µL SUPERNATANT INTO NEW COLUMNS 3 & 4
p300.transfer(800, deep_well.rows()[0][0], deep_well.rows()[0][2], mix_after=(3, 50))
p300.transfer(800, deep_well.rows()[0][1], deep_well.rows()[0][3], mix_after=(3, 50))

# PAUSE FOR 1 HOUR FOR SALTS TO SETTLE
protocol.delay(minutes = 60)
```

★ Liquid uptake not uniform → change flow rate

★ Avoid centrifuged pellet / NaOH salts → increase z height

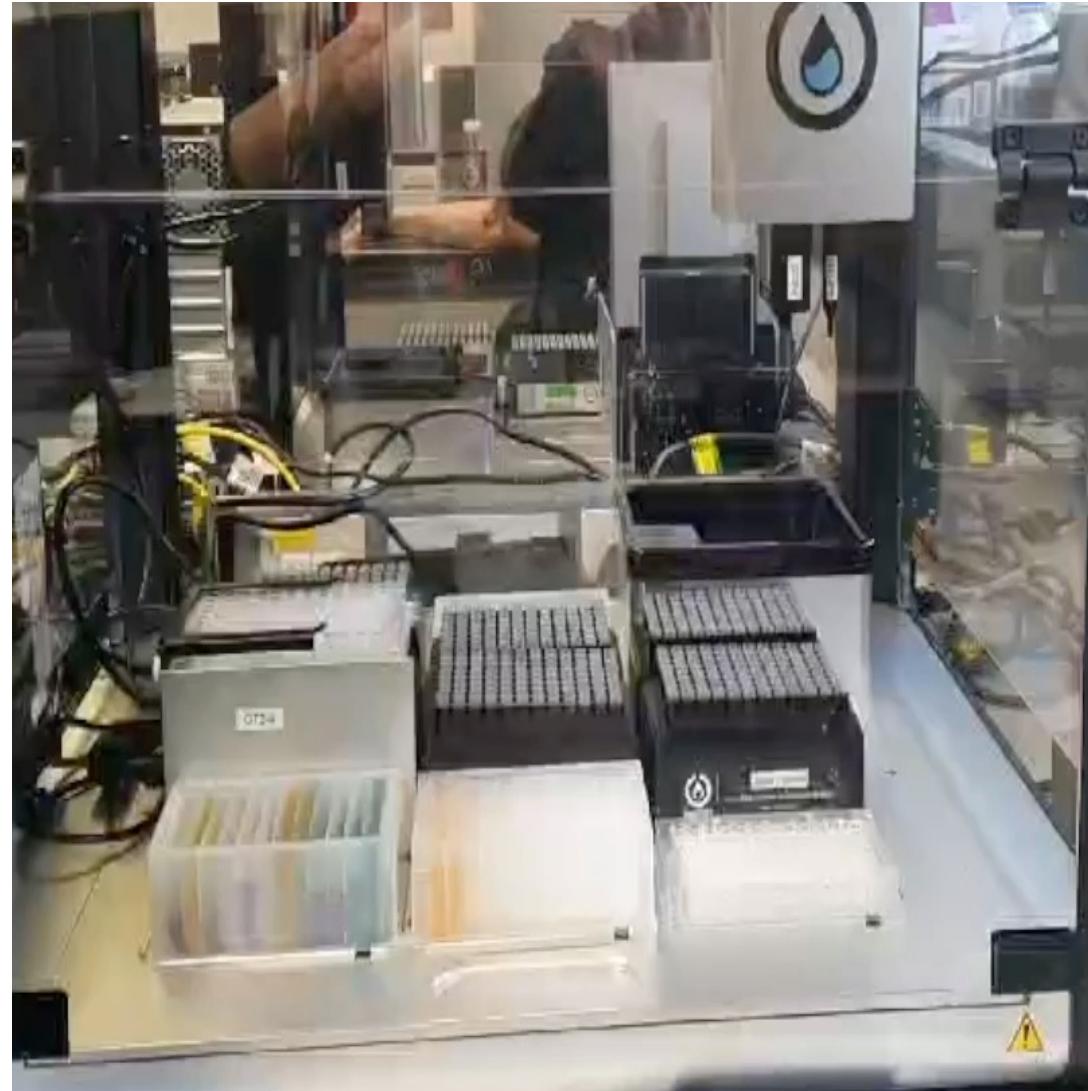
```
# DEFINE PIPETTE FLOW RATE (default is 94, decreased to aid liquid uptake)
p300.flow_rate.aspirate = 40
# DEFINE HOW FAR DOWN PIPETTE GOES (default is 1 mm from bottom of well, increased to 5 to aid uptake & avoid disruption of
centrifugation pellet / magnetic beads)
p300.well_bottom_clearance.aspirate = 5
p300.well_bottom_clearance.dispense = 5
```

Sample prep workflow

Prep

Ni-NTA

ELISA



Purification

Prep

Ni-NTA

ELISA

1 & 2. Equilibrate beads, bind samples

★ Deep plate doesn't fit on module → used 96 well plate & split sample

```
# TAKE TWO 300 µL ALIQUOTS OF NEUTRALISED SAMPLES
# magnetic plate wells cannot hold 600 µL so divide them up
# leaves 280 µL of neutralised samples in slot 2, cols 3 & 4
# samples 1-8 made into two tech reps in cols 5,6
p300.transfer(300, deep_well.rows()[0][2], deep_well.rows()[0][4:6])
#samples 9-16 made into two tech reps in cols 7,8
p300.transfer(300, deep_well.rows()[0][3], deep_well.rows()[0][6:8])
```

★ Strong magnet, affects protein-bead binding → move off magnet

```
# TRANSFER 300 uL OF SAMPLES ONTO MAG PLATE TO MIX WITH BEADS AND GIVE ENOUGH VOLUME TO REMOVE BEADS
# TRANSFER SAMPLE+BEAD MIX BACK TO DEEP WELL PLATE (magnet very strong so this maximises protein binding)
# samples 1-8
p300.transfer(300, deep_well.rows()[0][4:6],mag_rack.rows()[0][1:3], mix_after=(3, 50))
p300.transfer(330, mag_rack.rows()[0][1:3],deep_well.rows()[0][4:6], mix_after=(3, 50))
# samples 9-16
p300.transfer(300, deep_well.rows()[0][6:8],mag_rack.rows()[0][1:5], mix_after=(3, 50))
p300.transfer(330, mag_rack.rows()[0][3:5],deep_well.rows()[0][6:8], mix_after=(3, 50))

# PAUSE FOR 5 MINUTES FOR PROTEIN TO BIND BEADS
protocol.delay(minutes = 5)
# TRANSFER BACK INTO MAGNETIC PLATE
p300.transfer(330, deep_well.rows()[0][4:6],mag_rack.rows()[0][1:3]), mix_after=(3, 50))
p300.transfer(330, deep_well.rows()[0][6:8],mag_rack.rows()[0][3:5]), mix_after=(3, 50))
```

Preparation of Ni-NTA beads

Prep

Ni-NTA

ELISA



Purification

Prep

Ni-NTA

ELISA

3. Wash twice

4. Elute protein

★ Strong magnet, affects elution → move off magnet

```
# ADD 100 µL ELUTION BUFFER
p300.transfer(100, reservoir["A5"], mag_rack.rows()[0][1:5], mix_after=(3, 50))
# TRANSFER OFF OF MAGNET TO MAXIMISE ELUTION (magnet very strong)
# samples 1-8 duplicates to deep well plate column 9
p300.transfer(100, mag_rack.rows()[0][1:3],deep_well.rows()[0][8], mix_after=(3, 50))
# samples 9-16 duplicates to deep well plate column 10
p300.transfer(100, mag_rack.rows()[0][3:5],deep_well.rows()[0][9], mix_after=(3, 50))
# PAUSE FOR 10 MINUTES FOR PROTEIN TO ELUTE
protocol.delay(minutes = 10)
# TRANSFER BACK TO MAGNETIC PLATE
# samples 1-8 to magnetic plate col 6, samples 9-16 to col 77
p300.transfer(200, deep_well.rows()[0][8:10],mag_rack.rows()[0][5:7], mix_after=(3, 50),new_tip = 'always')
# ENGAGE MAGNET
mag_mod.engage(height_from_base=4.4)
# PAUSE FOR 2 MINUTES FOR BEADS TO SETTLE
protocol.delay(minutes = 2)
# TRANSFER 200 µL ELUTED SAMPLE INTO DEEP WELL PLATE COLS 11 & 12
p300.transfer(200, mag_rack.rows()[0][5:7], deep_well.rows()[0][10:])
```

ELISA



1. Serially dilute purified samples with coating solution 2x to 64x

- ★ Normal 96 well plate → changed z height back
- ★ Each sample needs coating buffer → no 1x dilution

```
# SET UP DILUTION PLATE - starting with 2x to 32x
# ADD COATING SOLUTION TO WELLS (cols 1-6, 7-12)
p300.transfer(100, reservoir["A6"], plate.rows()[0][0:12])

# TRANSFER 100 µL PURIFIED SAMPLES 1-8 TO ELISA PLATE COLUMN 1
# (100 µL purified samples remain in deep well cols 11 & 12 for reserve)
p300.transfer(100, deep_well.rows()[0][10], plate.rows()[0][0])#, mix_after=(3, 50))
# TRANSFER 100 µL PURIFIED SAMPLES 9-16 TO ELISA PLATE COLUMN 7
p300.transfer(100, deep_well.rows()[0][11], plate.rows()[0][6])#, mix_after=(3, 50))

# READJUSTING THE ASPIRATE AND DISPENSE HEIGHTS TO THE DEFAULT NOW THAT DONE WITH DEEP WELL
p300.well_bottom_clearance.aspirate = 1
p300.well_bottom_clearance.dispense = 1

# MAKING DILUTIONS OF SAMPLES 1-8 FROM COLUMN 1 TO 6
p300.transfer(100, plate.rows()[0][:5], plate.rows()[0][1:6], mix_after=(3, 50))
# EMPTY THE EXTRA 100 uL FROM COLUMN 6 so 100 µL in all wells
current_trash += 800
p300.transfer(100, plate.rows()[0][5], trash[which_trash(current_trash)])#, mix_after=(3, 50))
```

ELISA – serial dilution

Prep

Ni-NTA

ELISA



ELISA



2. Add blocking buffer, PBS wash x3

```
# ADD 200 µL BLOCKING BUFFER
p300.transfer(200, reservoir["A7"], plate.rows()[0][11::-1])
# PAUSE FOR 1 HOUR FOR BLOCKING SOLUTION TO BIND
protocol.delay(minutes=60)
# REMOVE BLOCKING BUFFER
current_trash += 1600*12
p300.transfer(200,plate.rows()[0][11::-1], trash[which_trash(current_trash)])
# WASH 3 TIMES WITH 100 µL PBS-T
for wash in range(3):
    current_PBS+=800*12
    p300.transfer(100,which_PBS(current_PBS,reservoir,trash) , plate.rows()[0][11::-1])
    current_trash += 800*12
    p300.transfer(100,plate.rows()[0][11::-1], trash[which_trash(current_trash)])
```

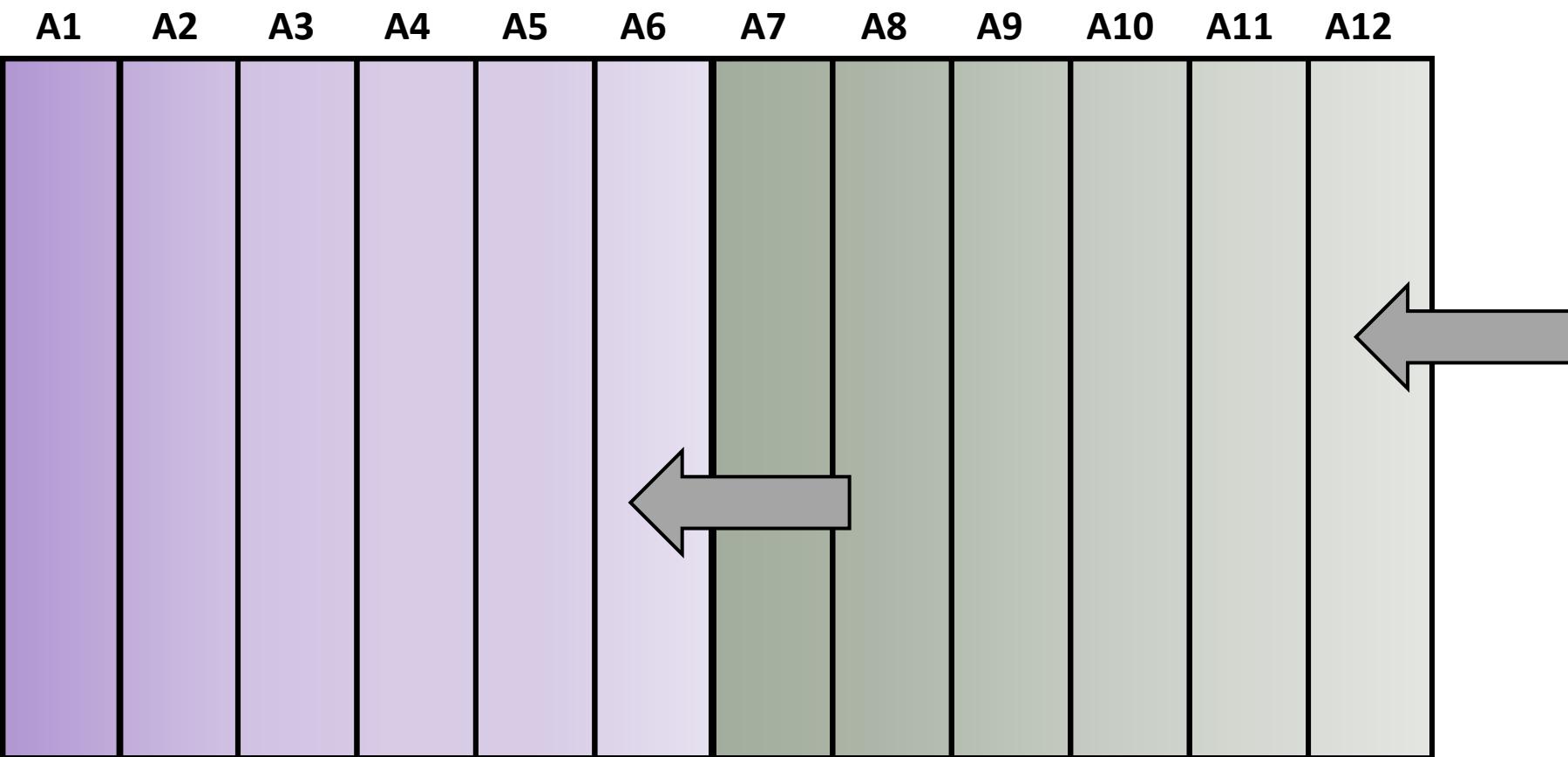
3. Add 1º antibody, PBS wash x3
4. Add 2º antibody, PBS wash x3
5. Add 1-step PnPP substrate
6. Stop reaction with NaOH
7. Measure absorbance (405 nm)

ELISA – minimising tip usage

Prep

Ni-NTA

ELISA



Remaining problems



Removed manual intervention steps

Shaking steps – no shaker modules

Invert plate and tap to remove excess liquid

Cover wells with cover slip



Specific use case

Could assign variables to reagent volumes, calculate in script



Scale limited by deck space / tips

Could split into two scripts (purification + ELISA)



Tip use

Reused tips for waste removal (ideally use clean)

Pipette could dispense over waste wells (avoids contamination)



ELISA standards – use previous data

If not limited by deck space, better to run standard alongside

Summary



- Outcome: fully automated protocol from raw samples to measuring absorbance
- Impact: saves time / labour and allows for overnight running
- Protocol implementation
 - Automation requires much more detailed protocols
 - Many problems overcome
 - Problems remain, not necessarily avoidable?

Thank you for listening!

