

- Announcements
- Quiz (last one!)
- Pre-lab Lecture
 - ❖ More about ELISA
 - ❖ DMMB assay
 - ❖ qPCR analysis
 - ❖ Today in lab (M3D6)

Announcements

- Lec 7: Atissa on proposal, discussion; Lec 8: special topics
- Next time: report + clean up, WAC + reflection surveys
- Mod 3 proposal
 - scope: breadth and depth aspects
 - pitfalls of choosing idea too early or too late
 - tips: talk with scientist(s) in the field, read reviews
- Mod 3 report
 - no separate methods section needed: state any unique conditions along the way in results section
 - required analysis: viability (incl. stats), qPCR (incl. RNA and cell counts), ELISA (incl. std. curves)
 - optional/if relevant analysis: PG assay; general bead, cell, media appearance

ELISA protocol

- Direct ELISA uses labeled primary antibody
- Indirect ELISA – why use a secondary antibody?

amplified signal
flexibility/efficiency (use w/many 1°AB)
but more cross-reactivity likely

- Development process – what/why/how

2° AB- has AP

provide substrate = PNPP \Rightarrow colorimetric rxn.
A₄₂₀

* development time is key \nearrow detect low conc.
 \searrow avoid over-saturation

ELISA Outcomes

| Outcome | Possible Explanations |
|---|---|
| High reading in "blank" samples | missed blocking step cross-contamination of wells * incomplete washing |
| No signal at all (including standards) | added wrong 2° Ab or <u>old</u> flipped plates @ 1° Ab too high [Tween] non-optimal development time |
| Saturated signal for some samples ↳ expt'l | too concentrated → run dilution series |

DMMB assay

- Measure GAGs with cationic dye
- Absorbance shift due to complex *Asgs!*
 - fades quickly! *(pipet upstairs)*
 - at low ^{*1.5*}pH, selects for *sulfates over carboxyls*
 - thus a correction for *alginate*
 - standard curve made with *chondroitin-6-sulfate*
- Typically normalize to cell amount (cf DNA content in qPCR)... maybe next year

qPCR analysis

$$\text{ratio} = \frac{(E_{\text{target}})^{\Delta C P_{\text{target}}(\text{control} - \text{sample})}}{(E_{\text{ref}})^{\Delta C P_{\text{ref}}(\text{control} - \text{sample})}}$$

Handwritten annotations:

- primer efficiency \rightarrow E_{target} and E_{ref}
- CNII or CNI \rightarrow $\Delta C P_{\text{target}}$ and $\Delta C P_{\text{ref}}$
- change in crossing point \rightarrow $\Delta C P_{\text{target}}$ and $\Delta C P_{\text{ref}}$
- 18S rRNA \rightarrow E_{ref}

control = sample A (-FN)

sample = sample B (+FN)

$E_{\text{target}} = 2.0$

$\Delta C P_{\text{target}} = 2$

$\frac{2^2}{2^0} = 4$

$E_{\text{ref}} = 2.0$

$\Delta C P_{\text{ref}} = 0$

fold-change

Equation 1 from M.W. Pfaffl, *Nucleic Acids Res* **29**:2002 (2001)

Today in Lab (M3D6)

- Finish ELISA – includes 90 min incubation
- Meanwhile...
 - DMMB assay staggered (15-20 min of work)
 - qPCR analysis
 - finish viability analysis if you haven't already
 - cross-group research discussion
 - (optional: start clean-up)