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

Announcements

- Lecture 7: Atissa on proposal, WAC evaluations
- 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, qPCR (incl. RNA), ELISA
  - optional/if relevant analysis: PG assay; general bead, cell, media appearance

next time: Y quiz, N notebooks

ELISA protocol

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

· amplify signal
flexibility/efficiency (w/many 1° Abs)
but more cross-reactivity likely

- Development process – what/why/how

2° Ab - has enzyme AP
provide substrate - p-NPP - colorimetric rxn.
↳ A420

* development time is key
↗ detect low conc
↘ avoid saturat.

ELISA Outcomes

Outcome	Possible Explanations
High reading in "blank" samples	cross-contamination of wells forgot to block incomplete washing
No signal at all (including standards)	absent or wrong specific plates e.g. step too high (Tween) non-optimal development time
Saturated signal for some samples	too concentrated → run dilution series

DMMB assay

- Measure GAGs with cationic dye
- Absorbance shift due to complex
  - fades quickly! (read upstairs)
  - at low pH, selects for sulfates over carboxyls
  - thus a correction for alginate
  - standard curve made with chondroitin 6 sulfate
- Typically normalize to cell amount (as DNA content)… maybe next year

qPCR analysis

primer efficiency

CN II or I

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

18S rRNA

* (1) control = culture A, sample = culture B (C1)

(2) control = stimulator CDR1, sample = A, B ...

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