

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

Announcements

- Assignment return plans
 - Mod 1 computational assignment Thu/Fri
 - Mod 2 revisions early next week
- Mod 3 report
 - No separate methods section needed
 - State any unique conditions along the way in results section or figure captions

ELISA protocol

- Direct ELISA uses labeled primary antibody
 - Indirect ELISA – why use a secondary antibody?
 - amplify signal
 - flexibility (use w/ many ¹⁰ Abs, more efficient)
 - but may be more cross-reactive
 - Development process – what/why/how
 - 2° Ab - has enzyme AP → Abs 420
 - provide substrate - PNPP - for colorimetric rxn.
- * development time is key → avoid saturation
↘ detect low conc.

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)	2° Ab went bad or incorrect non-optimal times too high (Titer)
Saturated signal for some samples. ↳ explicit	too concentrated → run a dilution series

DMMB assay

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

qPCR analysis

amplification efficiencies of primer sets

CNT I or II

- ① control - culture 1
Sample - culture 2
- ② control - fresh cell
Sample culture 1 ; 2

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

10S rRNA

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
 - cross-group research discussion (or another day)
 - finish viability analysis if you haven't already