- Announcements
- Quiz
- Pre-lab Lecture
  - More about ELISA

Announcements

- Final presentations May 12th/13th
  - Room 16-336
  - Start at 1:30 pm sharp
- Plan for Thursday, May 14th
  - Lecture: give feedback, then fill out evaluations
  - Afterward, lab party in 56-614, 12:30-2pm (RSVP)

ELISA Outcomes

Outcome	Possible Explanations
High reading in "blank" samples	* incomplete washes forgot block step
No signal at all (including standards)	• wrong conc. of soap (↑) • wrong 2° Ab • reagents old
Saturated signal for some samples	• too concentrated, repeat w/dilution series

ELISA protocol

- Direct ELISA uses labeled primary antibody
- Indirect ELISA – why use a secondary antibody?
  - flexibility (use many 1° Abs, more efficient time/cost)
  - amplification
  - ∴ but cross-reactivity is more likely
- Sandwich ELISA antibody selection

  Capture – polyclonal detection – monoclonal
- Development process – what/why/how

  2° – enzyme (AP) provide substrate p-NPP colorimetric rxn.
  - turn on @ desired time
  - bio./gentle conditions
  - amplify signal → dev. time is key