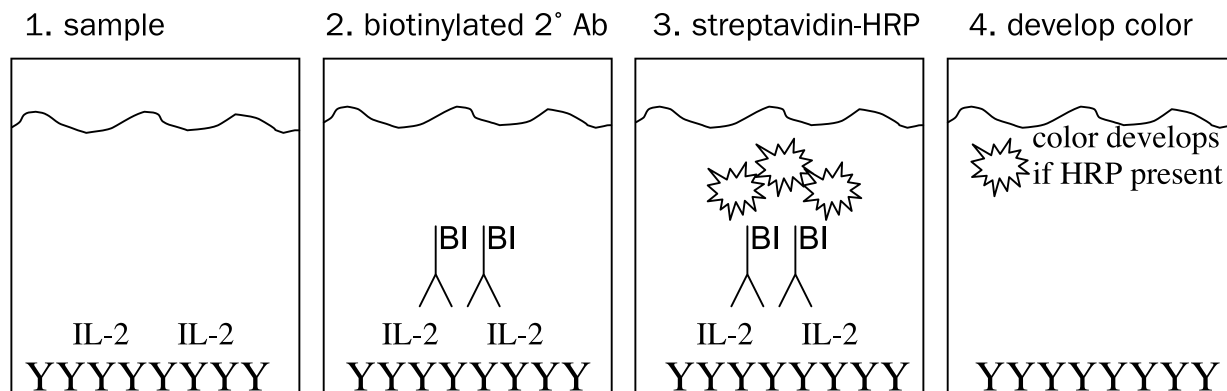
Colby College BIOLOGY 225 Spring 2013
Prof. Hannum

Lab 5
Quantitative IL-2 ELISA

ELISA is used not only to detect the presence of certain antigens in a sample, but also to quantitate the amount of antigen present. In this week's ELISA lab, we'll determine the amount of IL-2 produced by T cells in the spleen cultures you set up last week.

Like our first ELISA, this will also be a sandwich assay. However, the secondary antibody is not conjugated directly to an enzyme that can cleave the developing solution to a colored product – we'll have to perform an extra step to accomplish this.

Today's secondary antibody is conjugated to **biotin**. Biotin is a molecule that binds extremely tightly to the molecule **streptavidin**. After the biotinylated secondary antibodies have bound to IL-2, you'll add streptavidin conjugated to the enzyme **HRP** (horseradish peroxidase). HRP is the enzyme that will then cause color development of our substrate.



<u>Prior to today's lab</u>

Yesterday, 96-well ELISA plates were coated with the capture antibody, anti-mouse IL-2. Only the LEFT half of each plate was coated – columns 7-12 have no capture antibody.

Earlier today, excess capture antibody was washed out of the wells, then wells were blocked with 1% BSA solution.

ELISA protocol To start, lab team should get:

an ELISA plate
IL-2 standard
your 5 frozen samples from last week
diluent solution (1% BSA in PBS)

**Note: it is essential that the wells of an ELISA plate never completely dry out. *Do not remove fluid from the wells until you are actually ready to put more fluid back in.*

1. Flick the liquid out of the wells of your ELISA plate into a sink. Wash the plate 3x with wash buffer from a squirt bottle. Leave the last wash in until you are ready to add your samples.

2. When you are ready to add samples, flick the last wash out of the wells, and bang the plate upside-down on paper towels to remove most of the remaining wash water.

3. Add 50 μl diluent solution (1% BSA) to wells in column 1-3, rows B-H

4. Add 100 μl of the IL-2 standard (at 6 ng/ml) to each well in column 1-3, row A

   Serial dilutions of IL-2 standard:

   - Put 3 tips on a multichannel pipetter set to 50 μl.
   - Carefully remove 50 μl from row A, then dispense it into row B
     Pipet up and down 4-5 times to mix (**don't make bubbles!!**)
   - Remove 50 μl from row B, dispense into row C and mix
   - Continue down plate until you have removed 50 μl from row H –
     now discard the solution remaining in your pipet tips.

5. Add 50 μl of your thawed samples to column 4-6, rows A, B, C, D and E. Refer to plate map for correct placement. Add 50 μl of 1% BSA to column 4-6 row F, as a negative control.

6. Incubate the plate at 37˚ C for 30 minutes.

7. When ready to add the secondary antibodies, wash plates 3x as before. Bang plate on paper towels.

8. Add 50 μl per well secondary antibody (biotinylated anti-mouse IL-2).

9. Incubate the plate at 37˚ C for 30 minutes.

10. Wash plate, then add 50 μl streptavidin-HRP per well.

11. Incubate 20 minutes.

12. Wash the plate 3x with wash buffer and add 50 μl developing substrate (TMB).

13. Once color has developed, add 50 μl stop solution (1M H_2SO_4). Plates will be read on the microplate reader at 405 nm.

Plate map

	1-3	4-6	7-9	10-12
A	IL-2 standard N	control supernatant 48 hours	X	X
B	1:2	ConA supernatant 24 hours	X	X
C	1:4	ConA supernatant 48 hours	X	X
D	1:8	beads supernatant 24 hours	X	X
E	1:16	beads supernatant 48 hours	X	X
F	1:32	Negative control (1% BSA solution)	X	X
G	1:64	(empty)	X	X
H	1:128	(empty)	X	X