

# Mod 2 Day 6: Analysis & Planning II

10/30/2013

- 1. Pre-lab Discussion, Mid-Term Evaluations**
2. Western Blot: Secondary antibody addition
3. Imaging the Western Blot (Licor Odyssey)
4. Tissue Culture: High Throughput Viability Assay

# Notes on Introductions & Reports

1. Motivating sentence required! Why should I read your paper?
2. What is the goal? Let this be your guide.
3. Logical progression.
4. Preview of the rest of the manuscript – hypothesis/methods/results/conclusions
5. Class-wide data

# Throwback:

## Mod 2 Day 4: Western Blot

4) Nitrocellulose transfer

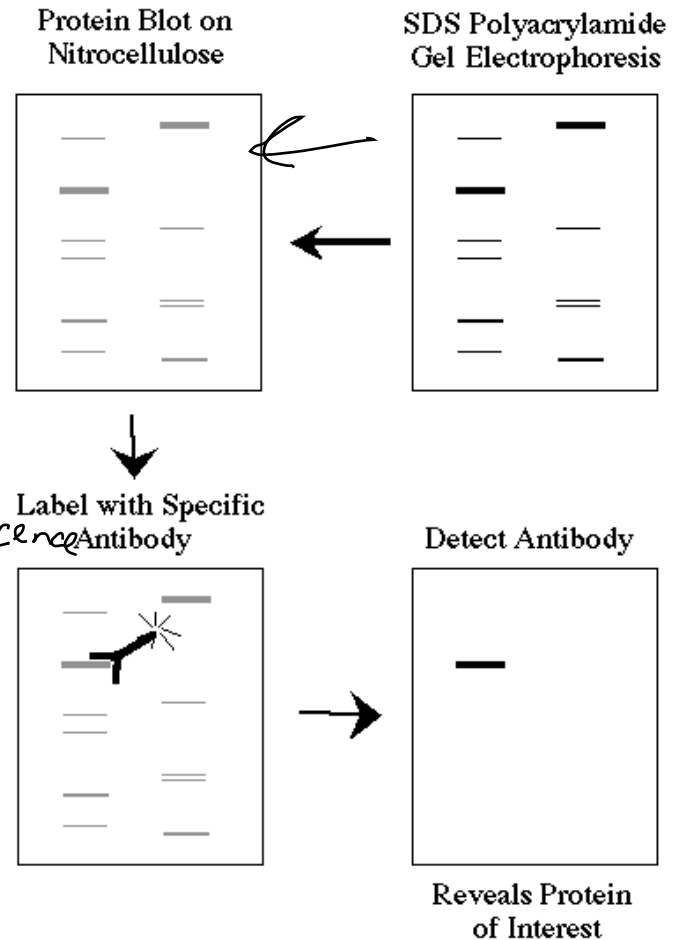
5) Probe w/ Ab's

Already done

- Blocking → slow auto fluorescence
- Primary Ab's

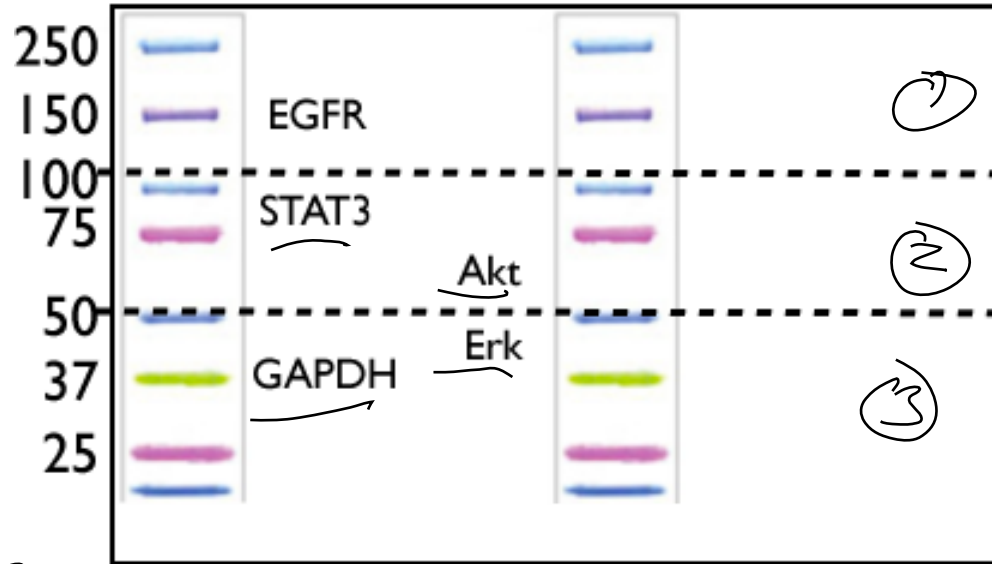
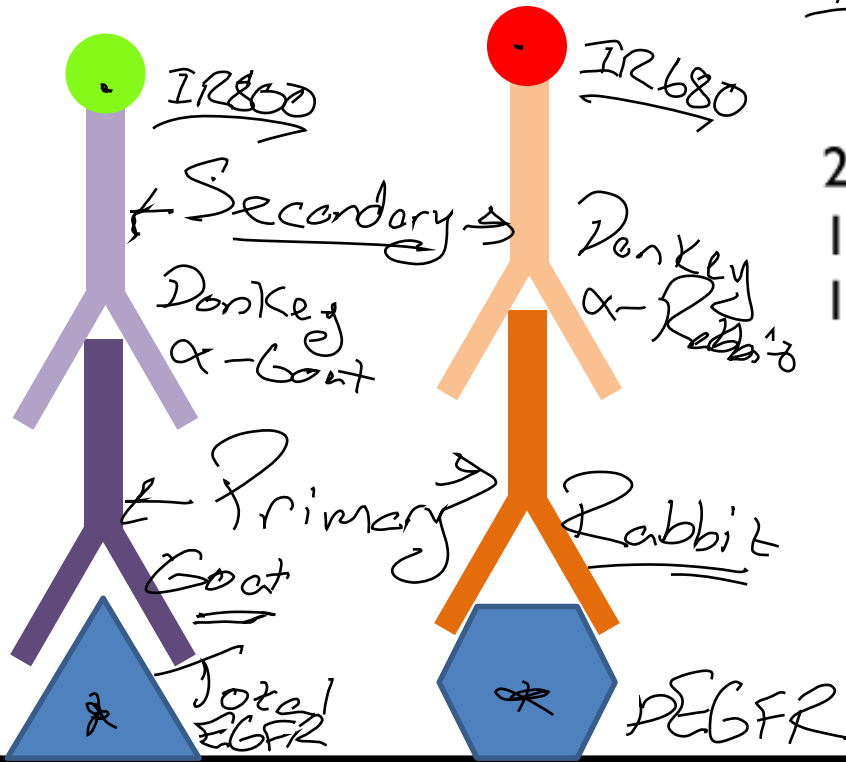
Today

- Secondary Ab
- Read



# Western Blot – Antibodies

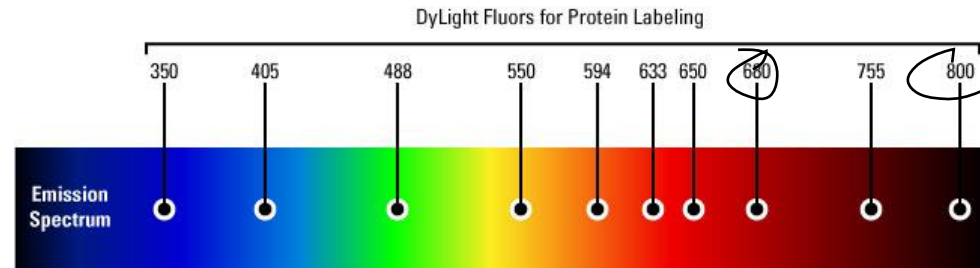
INDIRECT → multiple targets



FAKE

Host-specific antibodies:

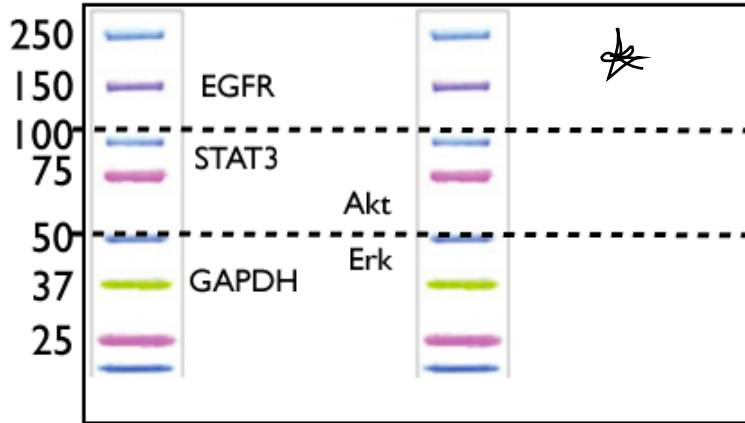
- Primary – goat anti-EGFR  
rabbit anti-pY1068-EGFR
- Secondary – donkey anti-goat IR800  
donkey anti-rabbit IR680



Click on a color above to jump to the respective DyLight Fluor labeling page  
Or see our new [DyLight Specialty Dyes](#) that span the length of the spectrum!

Modified

# Western Blot – Antibodies



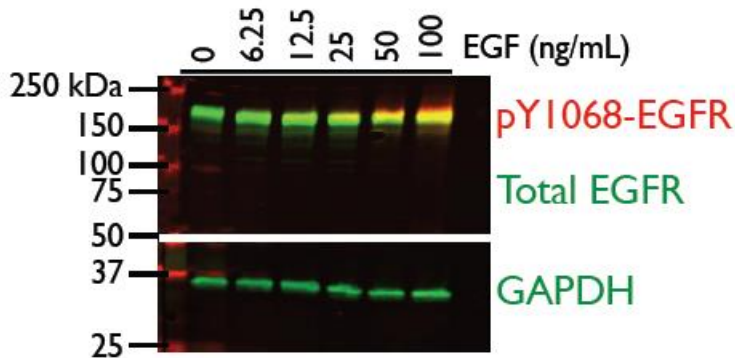
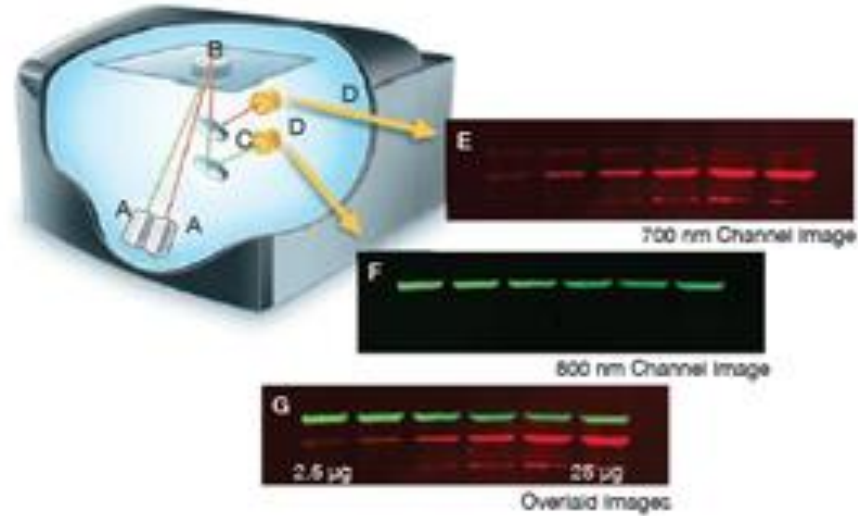
← Only one stripped!

Akt  
 Total → rabbit  
 so Strip Ab's  
 0.2M NaOH  
 THEN Total Akt

Primary Ab	Species	Secondary Ab	Akt?	Erk?	STAT3?
EGFR	Goat	anti-Goat IR800	★	★	★
p-EGFR	Rabbit	anti-Rabbit IR680	★	★	★
GAPDH	Rabbit	anti-Rabbit IR800	★		★
p-Akt	Rabbit	anti-Rabbit IR800	★		
Total Akt	Mouse	anti-Mouse IR680	★		
p-Erk	Rabbit	anti-Rabbit IR800		★	
Total Erk	Mouse	anti-Mouse IR680		★	
p-STAT3	Rabbit	anti-Rabbit IR800			★
STAT3	Mouse	anti-Mouse IR680			★

# Read on Licor Odyssey

Inverted microscope/camera  
Lasers to excite fluor.  
Store for a long time

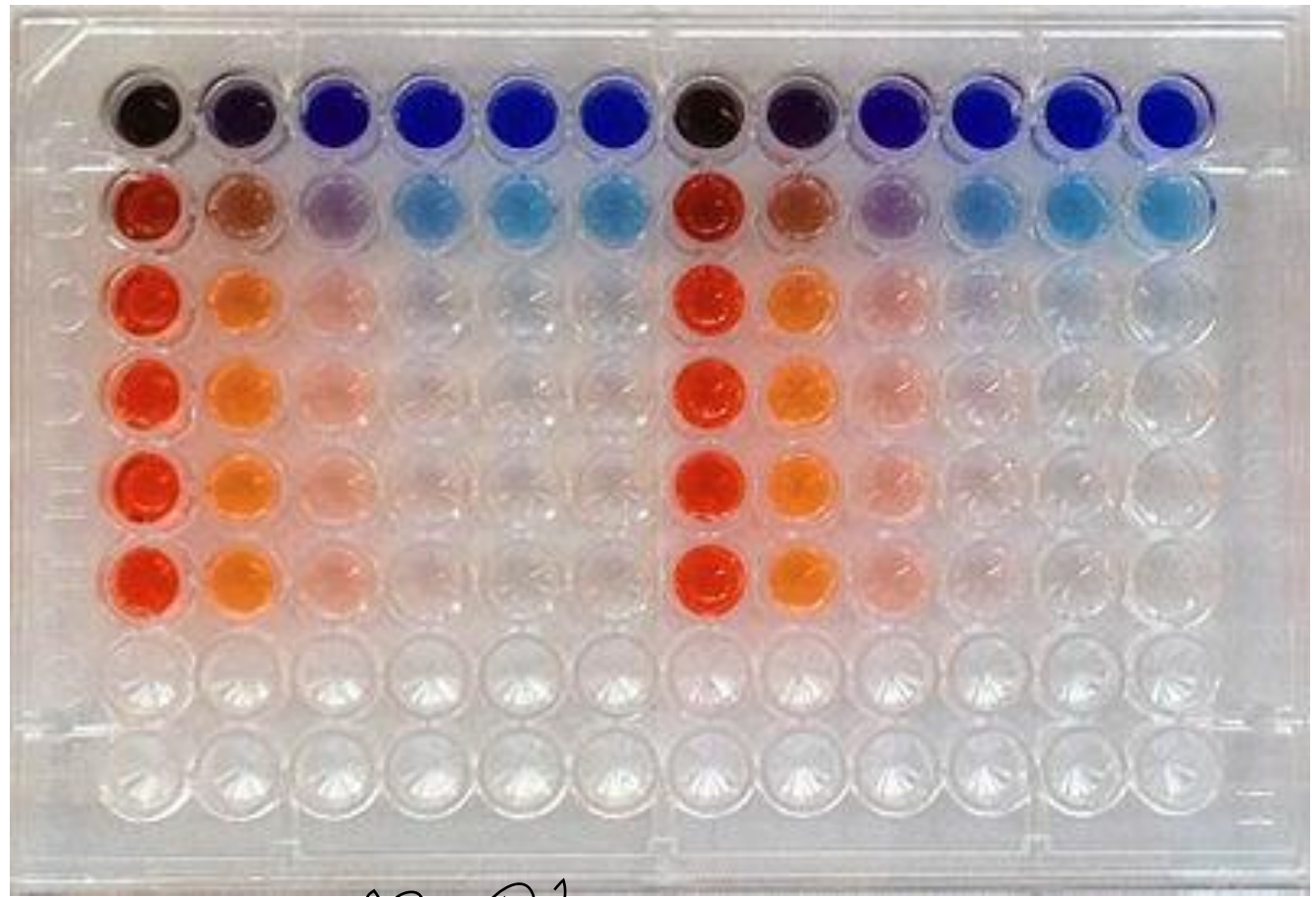


- ① Remove primary
- ② Wash 3x TBS-T  
T = Tween 20  
10 min
- ③ ~~Remove~~ Secondary Ab \*  
2 min wash
- ④ Wash TBS-T 3x  
2 min
- ⑤ PBS

# High-Throughput Viability Assay

(your choice inhib.)  
Inhibitor X  $\xrightarrow{\text{Diluted}}$  RED

Erlotinib  
↓  
Blue



BUT, everything will be red this time (b/c media)

MZD1

# Set-up Plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	E20	E20	E20	E20	E20	E20	X1	M	M	M	M	M+D
B	E2	E2	E2	E2	E2	E2	X1	M	M	M	M	M+D
C	E0.2	E0.2	E0.2	E0.2	E0.2	E0.2	X1	M	M	M	M	M+D
D	E0.02	E0.02	E0.02	E0.02	E0.02	E0.02	X1	M	M	M	M	M+D
E	E.002	E.002	E.002	E.002	E.002	E.002	X1	M	M	M	M	M+D
F	E0	E0	E0	E0	E0	E0	X1	M	M	M	M	M+D
G	PC	EGF 50	EGF 25	EGF 12.5	EGF 6.25	EGF 0						
H	PC	EGF 50	EGF 25	EGF 12.5	EGF 6.25	EGF 0						

m = McCoy's media  
 + 1% Serum  
 + 12.5 ng/mL EGF

multichannel  
 for dilutions

Regular  
 m+D → media + 0.001% DMSO  
 E0/X1 → Erlotinib [X] ↑  
EGF dilutions

Pos Control  
 Kills cells!  
 Media + 1% DMSO

Reg cellular resp  
 to how much we  
 activate EGF<sub>R</sub>  
 pathway  
 HALF! 20µm Erlotinib

Final concentrations will be...

HALF! 20µm Erlotinib



# Experimental Plate

*Change* →

*Same tips* ↑

	1	2	3	4	5	6	7	8	9	10	11	12
A	E10 X1	E10 X2	E10 X3	E10 X4	E10 X5	E10 X0	E10 X1	E10 X2	E10 X3	E10 X4	E10 X5	E10 X0
B	E1 X1	E1 X2	E1 X3	E1 X4	E1 X5	E1 X0	E1 X1	E1 X2	E1 X3	E1 X4	E1 X5	E1 X0
C	E0.1 X1	E0.1 X2	E0.1 X3	E0.1 X4	E0.1 X5	E0.1 X0	E0.1 X1	E0.1 X2	E0.1 X3	E0.1 X4	E0.1 X5	E0.1 X0
D	E0.01 X1	E0.01 X2	E0.01 X3	E0.01 X4	E0.01 X5	E0.01 X0	E0.01 X1	E0.01 X2	E0.01 X3	E0.01 X4	E0.01 X5	E0.01 X0
E	E.001 X1	E.001 X2	E.001 X3	E.001 X4	E.001 X5	E.001 X0	E.001 X1	E.001 X2	E.001 X3	E.001 X4	E.001 X5	E.001 X0
F	E0 X1	E0 X2	E0 X3	E0 X4	E0 X5	E0 X0	E0 X1	E0 X2	E0 X3	E0 X4	E0 X5	E0 X0
G	PC	EGF 50	EGF 25	EGF 12.5	EGF 6.25	EGF 0	PC	EGF 50	EGF 25	EGF 12.5	EGF 6.25	EGF 0
H	PC	EGF 50	EGF 25	EGF 12.5	EGF 6.25	EGF 0	PC	EGF 50	EGF 25	EGF 12.5	EGF 6.25	EGF 0

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- ☞ **Cell seeding density: 31,250 cells/cm<sup>2</sup>, 16 hours on plate**  
Multichannel pipette for everything here!

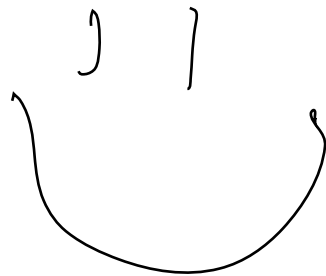
# Hints

- Have everything ready before media is removed from cells
- Do not change aspirating pipette/wash with ethanol between wells. Do not touch cells.  
*Leave a bit of media*
- “Purposeful pace”!

# Next time

- CellTiter-Glo
  - Add CTG reagent to lyse cells
  - Incubate for 20 minutes
  - Measure luminescence signal

Done!



# Today!

- Tissue Culture:  
Orange, Yellow, Purple
- Western Blot:  
White, Pink, Green, Red, Blue

## Mod 2 Day 7:

- CellTiter-Glo assay (here or in Koch)
- Analyze with Matlab/Excel on personal computer  
(can be done in lab or on your own time)
- M2D6 FNT due Sunday, Nov 2<sup>nd</sup> at 5pm to Stellar!

