M2D6: Analysis & Planning II

10/29/13

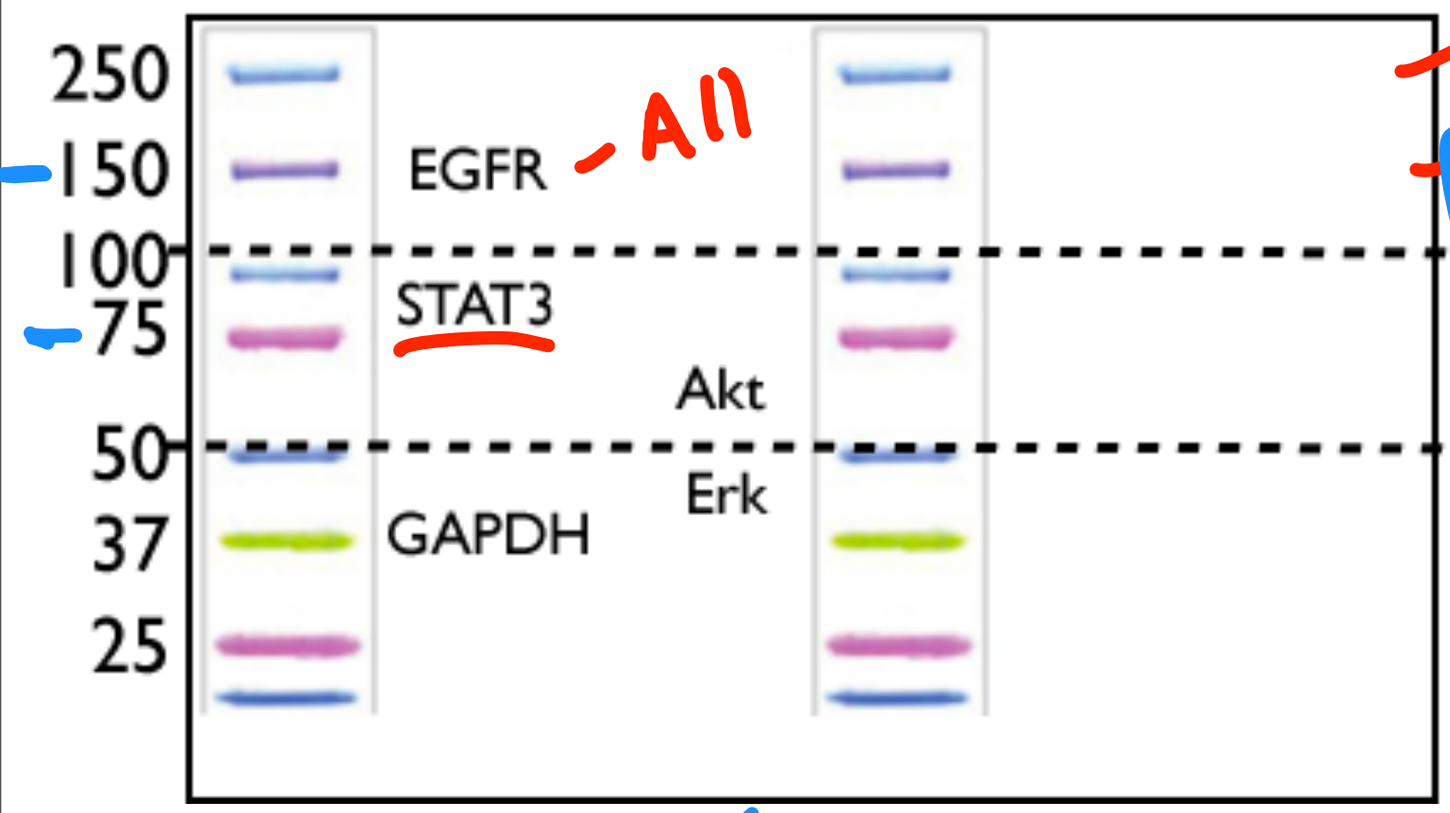
1. Pre-lab discussion -- re: mid-term evals
2. Notes about Introductions
3. Set-up cell viability HTS -- Orange, Green, Pink, Platinum
4. 2nd part of WB analysis -- White, Yellow, Purple, Blue, Red
5. Upcoming deadlines

Notes on Introductions (& Mod2 report)

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.

Western blot analysis -- part II

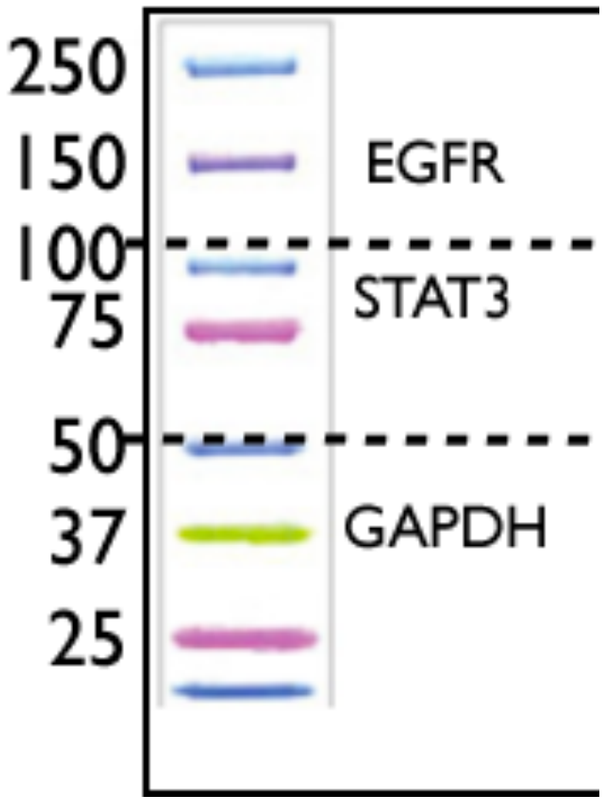
Kal.

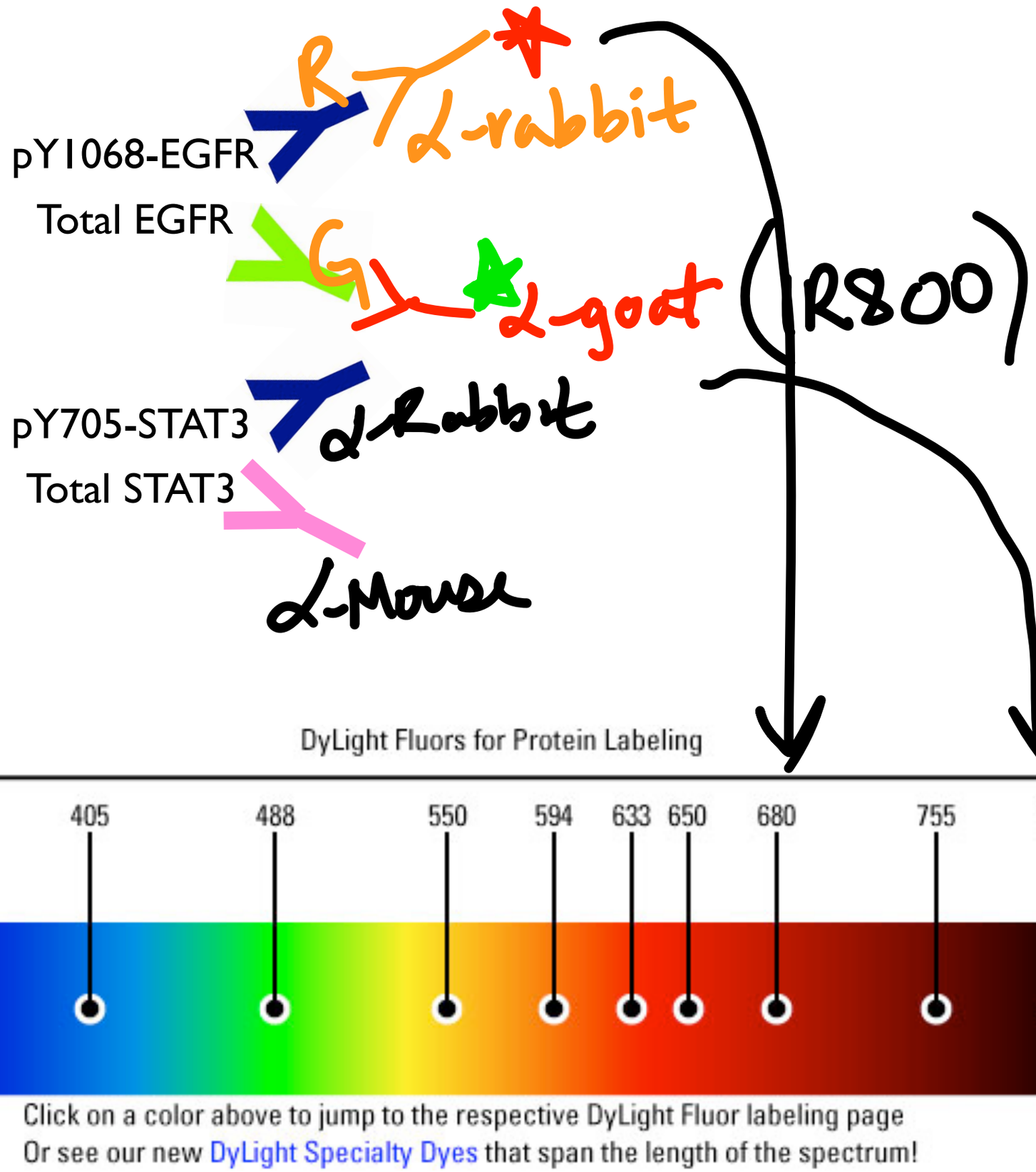


- All
- pEGFR
- tEGFR
- goat
- pY705 STAT3
- ★ Akt
- Today. pAkt
- Tonight: Strip
- 0.2N NaOH
- reprobe w/total Akt

			Did your team choose to inhibit:		
Antibody	Species	Approx. MW	Akt pathway?	Erk pathway?	STAT3 pathway?
EGFR	Goat	150 kDa	X	X	X
tyrosine 1068 pY1068-EGFR	Rabbit	150 kDa	X	X	X
GAPDH	Rabbit	37 kDa	X		X
pS473-Akt	Rabbit	64 kDa	X		
total Akt	Mouse	64 kDa	X		
pT202/pY204-Erk	Rabbit	42/44 kDa		X	
total Erk	Mouse	42/44 kDa		X	
pY705-STAT3	Rabbit	75 kDa			X
total STAT3	Mouse	75 kDa			X

Western blot analysis -- part II

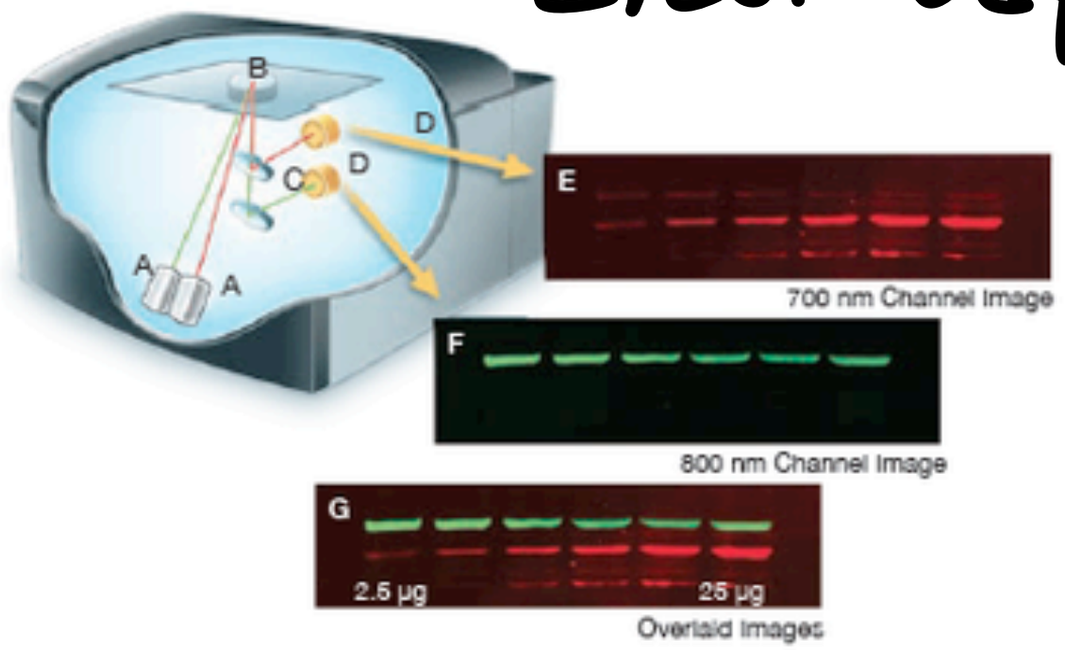


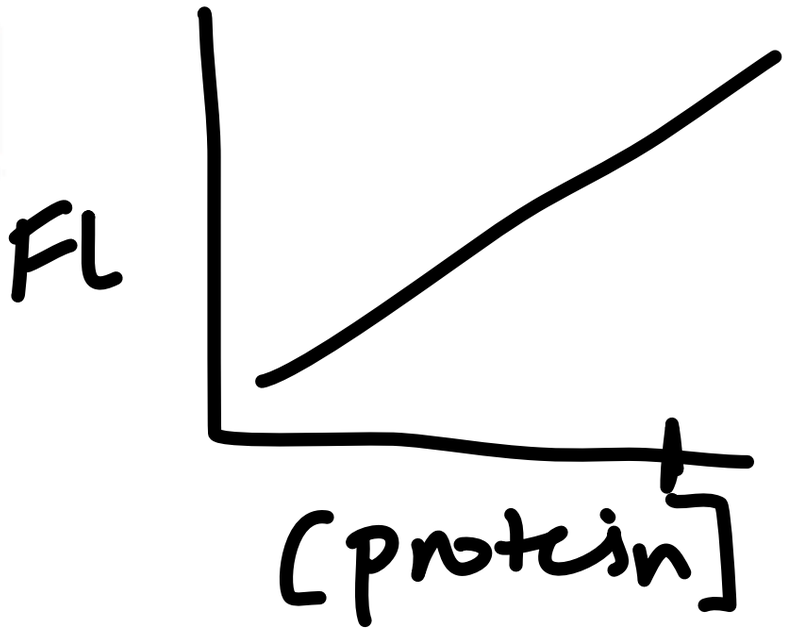


http://www.piercenet.com/cat/dylight-fluor-labeling-reagents-kits

Western blot analysis -- part II

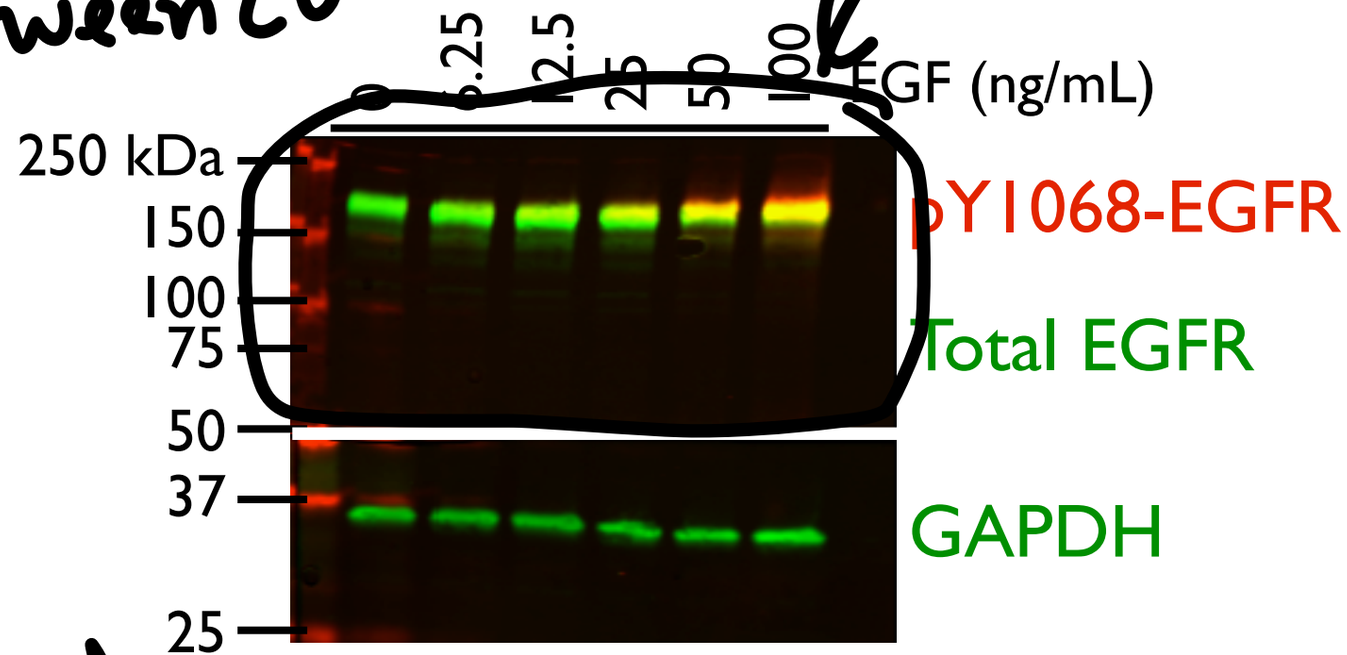
Licor Odyssey



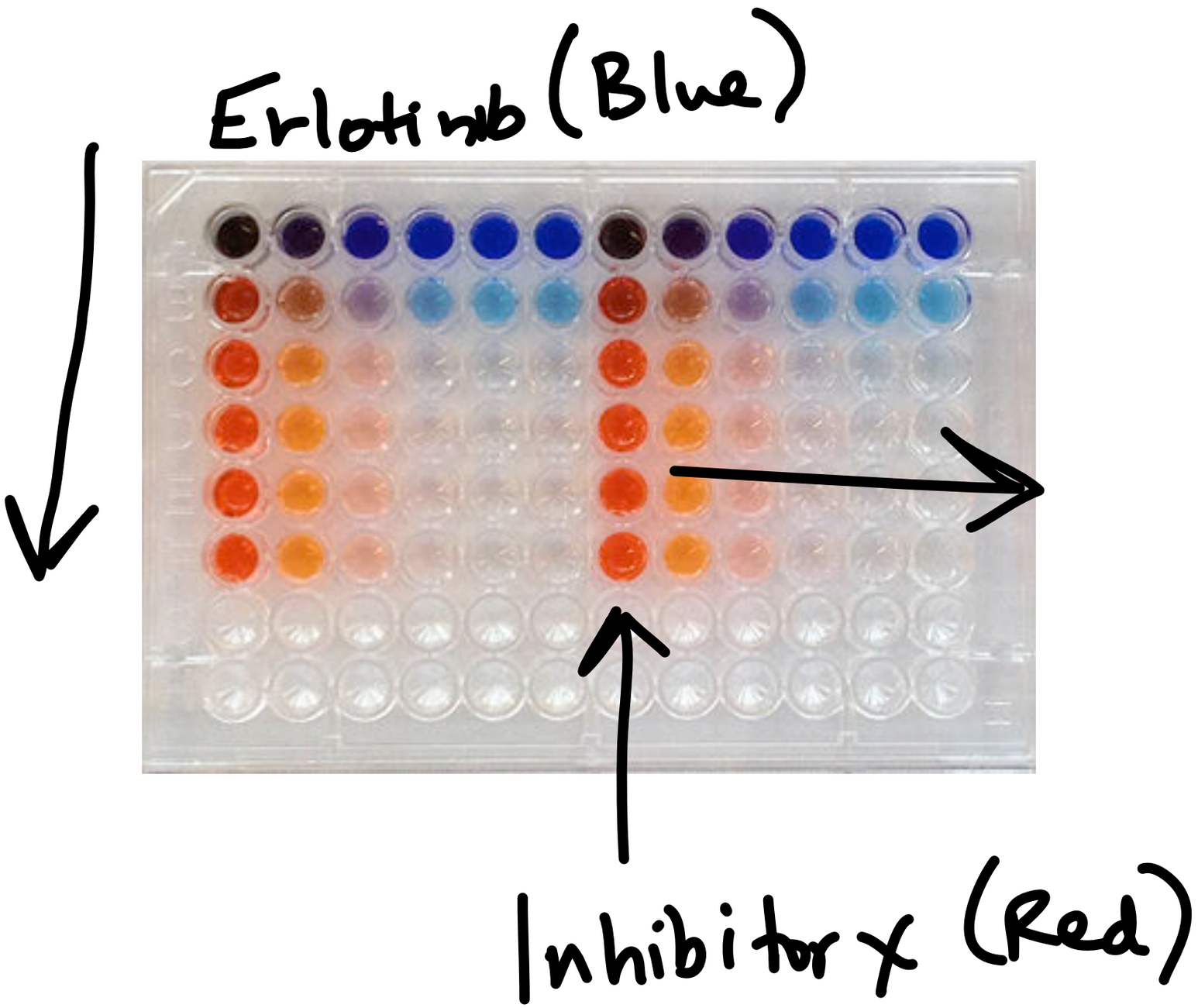


- remove 1° Ab
- Wash 3 x TBS-T ← tween20
- 2°Ab - 30min
- Wash TBS-T
- PBS ← autofl. background

memory stick



Set-up viability analysis screen:



PC = M + 1% DMSO

'Dilution' plate:

* use multichannel

M = media

	1	2	3	4	5	6	7	8	9	10	11	12
A	E20	E20	E20	E20	E20	E20	X1	M X2	M X3	M X4	M X5	M+D X0
B	E2 M	E2 M	E2 M	E2 M	E2 M	E2 M	X1	M X2	M X3	M X4	M X5	M+D X0
C	E0.2 M	E0.2 M	E0.2 M	E0.2 M	E0.2 M	E0.2 M	X1	M X2	M X3	M X4	M X5	M+D X0
D	E0.02 M	E0.02 M	E0.02 M	E0.02 M	E0.02 M	E0.02 M	X1	M X2	M X3	M X4	M X5	M+D X0
E	E.002 M	E.002 M	E.002 M	E.002 M	E.002 M	E.002 M	X1	M X2	M X3	M X4	M X5	M+D X0
F	E0 M+D	E0 M+D	E0 M+D	E0 M+D	E0 M+D	E0 M+D	X1	M X2	M X3	M X4	M X5	M+D X0
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						

McCoys +
1% serum
+
12.5 ng/mL EGF

M+D – DMSO
E20/X1
EGF dilutions

* regular pipette

Final concentration will be: 10 μM Erl
20 μM Stattic

Where to use multi-channel pipette: ✓

‘Experimental’ plate:

Cell seeding density: 31,250 cells/cm^2, approx. 16 hr on plate

	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

NO cells

There are no cells in:

Where to use multi-channel pipette: Everything

‘Experimental’ plate:

Hints:

1. Have everything ready to go before removing media from cells.

2. Do not change aspirating pipette or wash with ethanol between wells. Don’t touch the cells!

3. Work at a purposeful pace -- this is a time to concentrate.

Preview of next time:

CellTiter-Glo

1) Add → CTG reagent – lyse

2) Incubate 20 min

3) Measure luminscent signal.

Today in the lab:

1. Orange, Green, Pink, Platinum -- to TC
2. White, Yellow, Purple, Blue, Red-- WB

Next time in the lab:

- Complete CellTiter-Glo assay -- here or in Koch
- Analyze data with Excel and Matlab -- bring your computer if possible. You can do this in lab or on your own time.
- ***M2D6 FNT is due on Sunday, Nov. 2nd at 5pm to Stellar.***