Alpha protein-protein interaction: Saturation curve experiment

1. Goal

- Create a saturation curve to determine Kd in Alpha assay

2. Reagents

Component	Vendor	Catalog number
EGFR-Fc	R&D Systems	#344-ER
Biotin-EGF	Invitrogen	#E-3477
Streptavidin Donor beads	PerkinElmer	#6760002S
Protein A AlphaLISA Acceptor beads	PerkinElmer	#AL101C
96-well ½ AreaPlate	PerkinElmer	#6005560
Assay buffer : PBS + 0.5% BSA	In-house	

3. Assay principle

This assay is designed to examine the interaction of epidermal growth factor (EGF, "the ligand") with its cognate receptor, epidermal growth factor receptor (EGFR, "the receptor"). EGF is a 53 amino acid small protein. Its discovery won Stanley Cohen the Nobel Prize in Medicine in 1986. EGFR is a receptor tyrosine kinase that is located on the cell surface. When EGF binds, it activates the tyrosine kinase activity of EGFR which begins a signaling cascade in the cell. This activity is frequently aberrant in cancer and inhibiting the EGFR response is an active area of research. This research field has spawned several clinical drugs including Tarceva (Genentech), Erbitux (ImClone/BMS), and Vectibix (Amgen).

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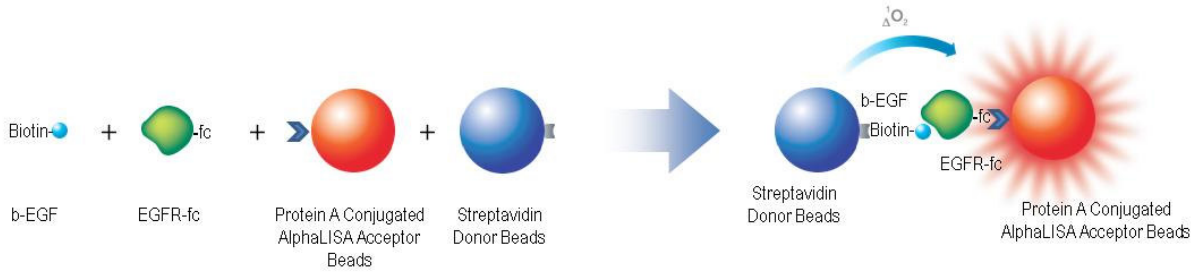

Figure 1. Alpha protein-protein assay design.

4. Reagent preparation

4.1 Preparation of EGFR-Fc (50 µg, MW=95.1 kDa):

a. Reconstitute EGFR-Fc in 0.5 mL PBS, to obtain 0.1 mg/mL (=100 µg/mL = 1.05 µM EGFR-Fc)
b. Dilute 1:10 in PBS + 0.5% BSA (100 µL EGFR-Fc + 900 µL [PBS + 0.5% BSA]) to get a 0.105 µM solution
c. Prepare three dilutions (intermediate concentrations: 12 nM, 4 nM, 1.2 nM):

Dilution	[Final] (M)	[Intermediate] (M)	Vol of dilution	Buffer (PBS + 0.5% BSA)
1	1×10^{-9}	4×10^{-9}	34 µL of 0.105 µM EGFR-Fc	858 µL
2	3×10^{-10}	12×10^{-10}	10 µL of 0.105 µM EGFR-Fc	865 µL
3	1×10^{-10}	4×10^{-10}	100 µL of dilution 1	900 µL

4.2 Preparation of Biotin-EGF (20 µg, MW~6300):

a. Reconstitute Biotin-EGF in 0.5 mL deionized water to obtain 40 µg/mL = 6.35 µM
b. Prepare a 400 nM dilution (63 uL of 6.35 µM biotin-EGF + 937 µL [PBS + 0.5% BSA])
c. Prepare dilutions in [PBS + 0.5% BSA]:

Dilution	[Final] (M)	[Intermediate] (M)	Vol of dilution	Buffer (PBS + 0.5% BSA)
A	15 nM	60 nM	30 µL of 400 nM stock	170 µL
B	10 nM	40 nM	63 µL of 400 nM stock	567 µL
C	5 nM	20 nM	40 µL of 400 nM stock	760 µL
D	2.5 nM	10 nM	250 µL of dilution C	250 µL
E	1 nM	4 nM	100 µL of dilution C	400 µL
F	0.5 nM	2 nM	250 µL of dilution E	250 µL
G	0.2 nM	0.8 nM	200 µL of dilution F	300 µL
H	0.05 nM	0.2 nM	100 µL of dilution G	300 µL

5. Prepare 4x working solution (80 µg/mL) of Protein A AlphaLISA Acceptor beads:

16 µL Acceptor beads (5 mg/mL) + 984 µL buffer (PBS + 0.5% BSA)

6. (During 2nd incubation): Prepare 4x working solution (80 µg/mL) of Alpha Streptavidin Donor beads:

16 µL Donor beads (5 mg/mL) + 984 µL buffer (PBS + 0.5% BSA)

7. Assay protocol for a 96-well ½ AreaPlate (Total assay volume of 40 µL)

Refer to plate map in section 8, on next page. You can use a multi-channel repeat pipettor to quickly dispense reagents into the plate.

Protein-protein interaction assay
1. Add 10 µL EGFR-Fc
2. Add 10 µL biotin-EGF
3. Incubate 60 min at room temperature
4. Add 10 µL Protein A Acceptor beads (final conc. 20 µg/mL)
5. Incubate 60 min at room temperature
6. Add 10 µL Streptavidin Donor beads (final conc. 20 µg/mL)
7. Incubate 30 min at room temperature
8. Read on an EnVision or EnSpire

8. Map for 96-well ½ AreaPlate (Samples in triplicate)

	1 nM EGFR-Fc			0.3 nM EGFR-Fc			0.1 nM EGFR-Fc			(empty)		
	1	2	3	4	5	6	7	8	9	10	11	12
A 15 nM biotin-EGF												
B 10 nM biotin-EGF												
C 5 nM biotin-EGF												
D 2.5 nM biotin-EGF												
E 1 nM biotin-EGF												
F 0.5 nM biotin-EGF												
G 0.2 nM biotin-EGF												
H 0.05 nM biotin-EGF												