

Improving the Throughput of Titer Determination for PER.C6[®] Cell Line Generation and Process Development with the Octet Platform

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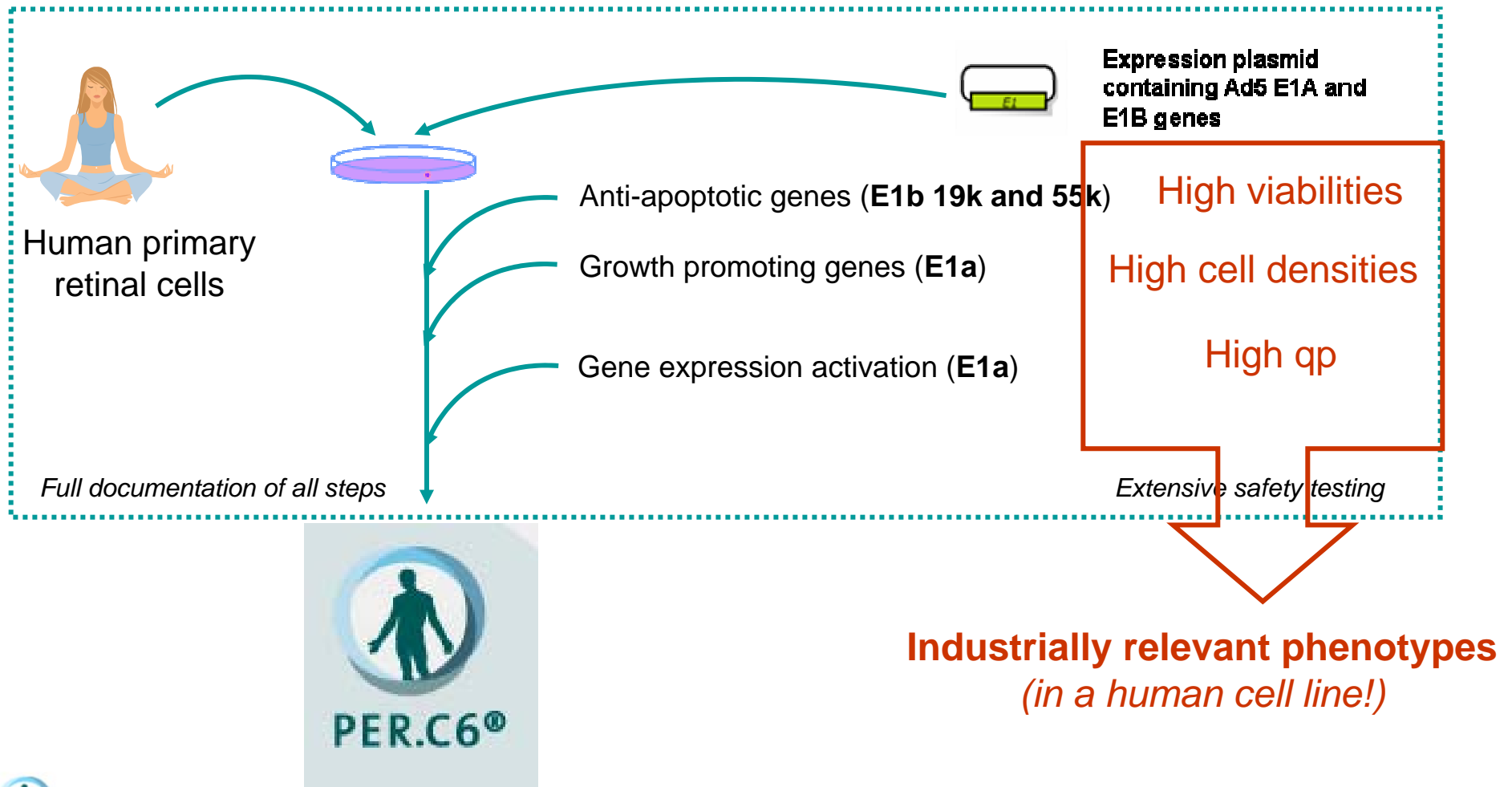
PERCIVIA, LLC, Cambridge, MA

Outline

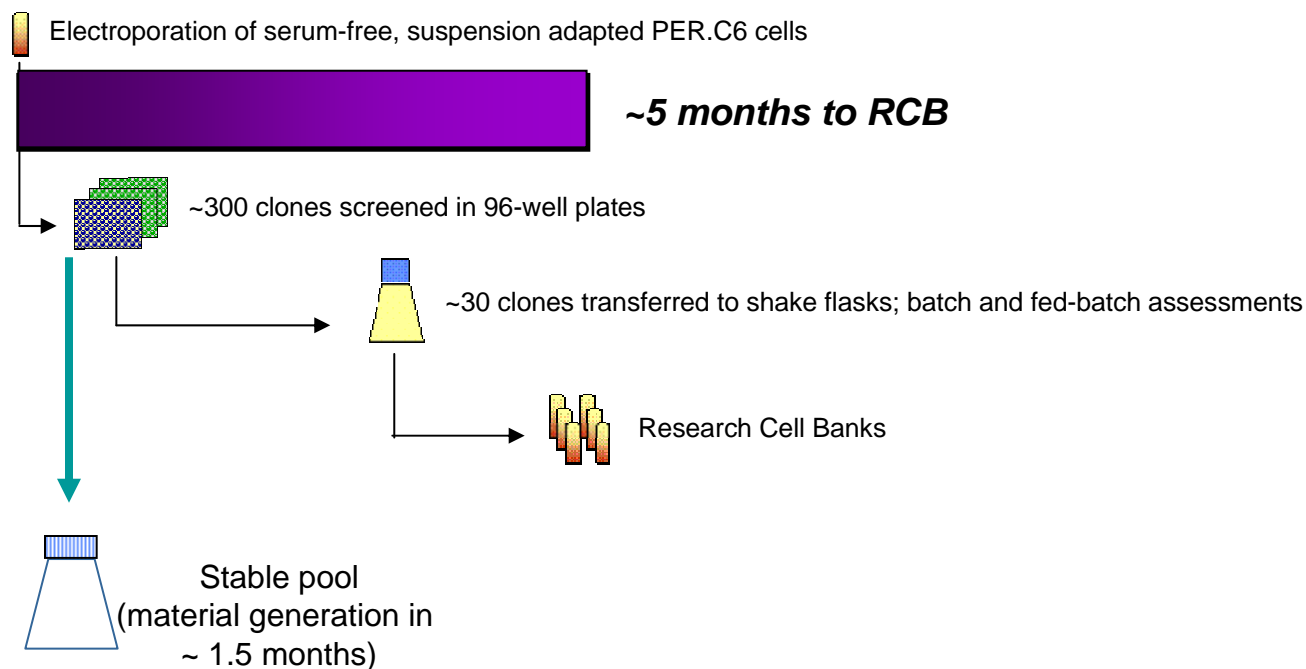
Delivery of a high throughput, automatable assay for titer evaluation to support clone selection and culture process development

- Introduction to PER.C6® technology platform
- Different titer methods
- FortéBio Octet
- Octet performance for IgG's
- Sensor regeneration
- Assay development for non-IgG's

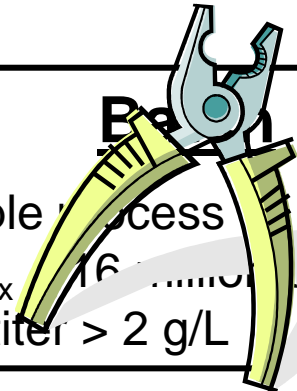
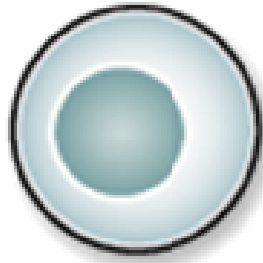
Biology of PER.C6[®] Cells



Cell line generation at PERCIVIA

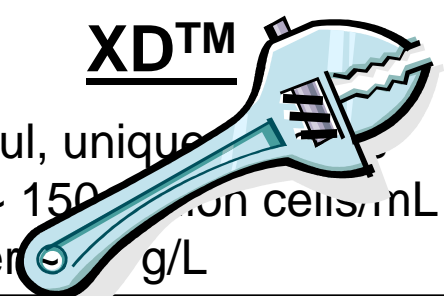


PER.C6® Platform Technology



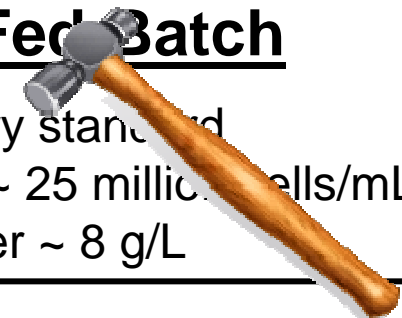
Batch

- Simple process
- Xv_{max} ~ 16 million cells/mL
- IgG titer > 2 g/L



XDTM

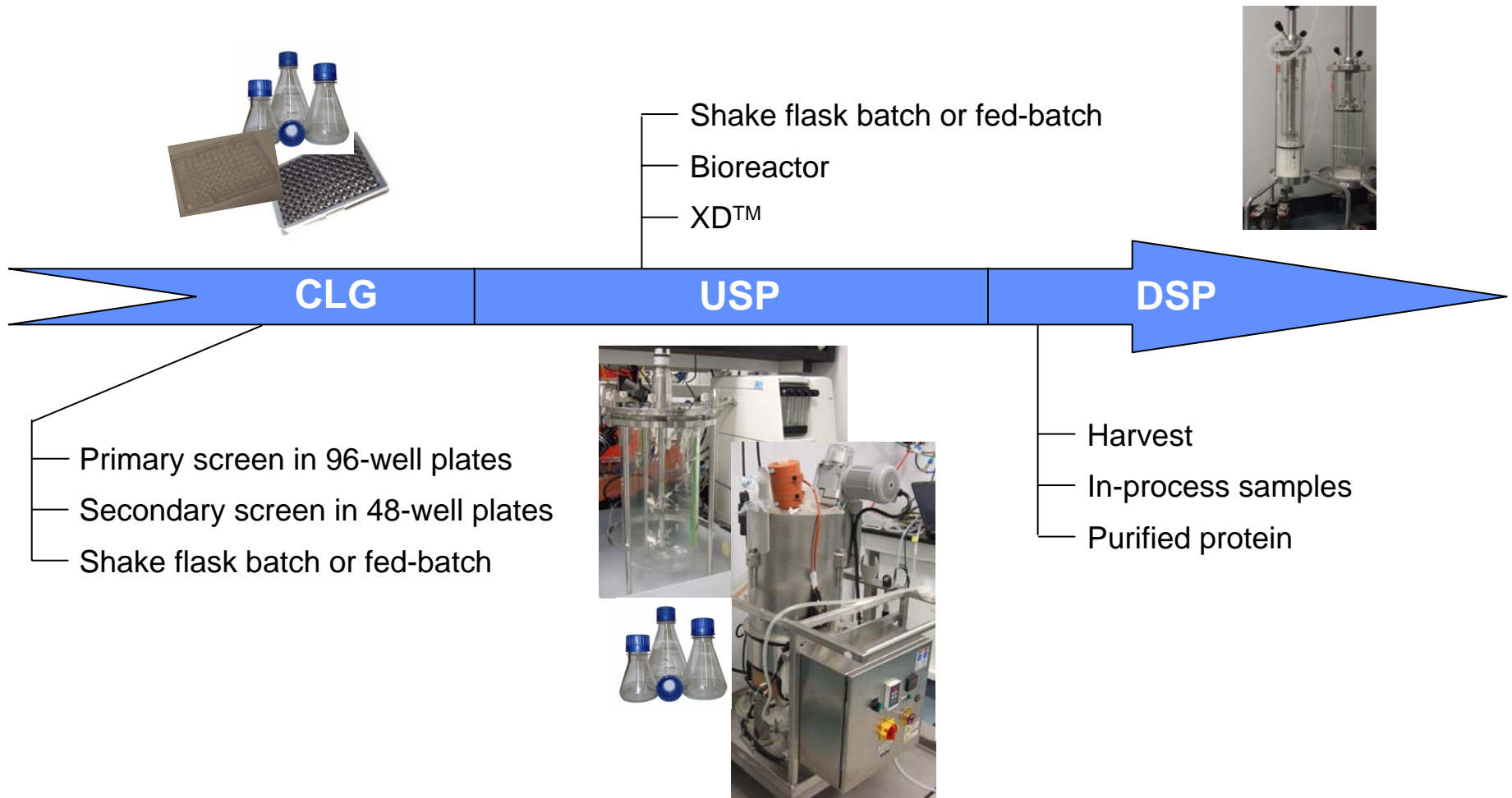
- Powerful, unique
- Xv_{max} ~ 150 million cells/mL
- IgG titer ~ 8 g/L



Fed Batch

- Industry standard
- Xv_{max} ~ 25 million cells/mL
- IgG titer ~ 8 g/L

Diversity of samples



Traditional IgG Titer assays

- Protein A HPLC assay
- ELISA

Assay	Limitations
ELISA	Lack of precision Lack of accuracy Low turnaround time
Prot A HPLC	Lack of sensitivity High sample volume Low throughput

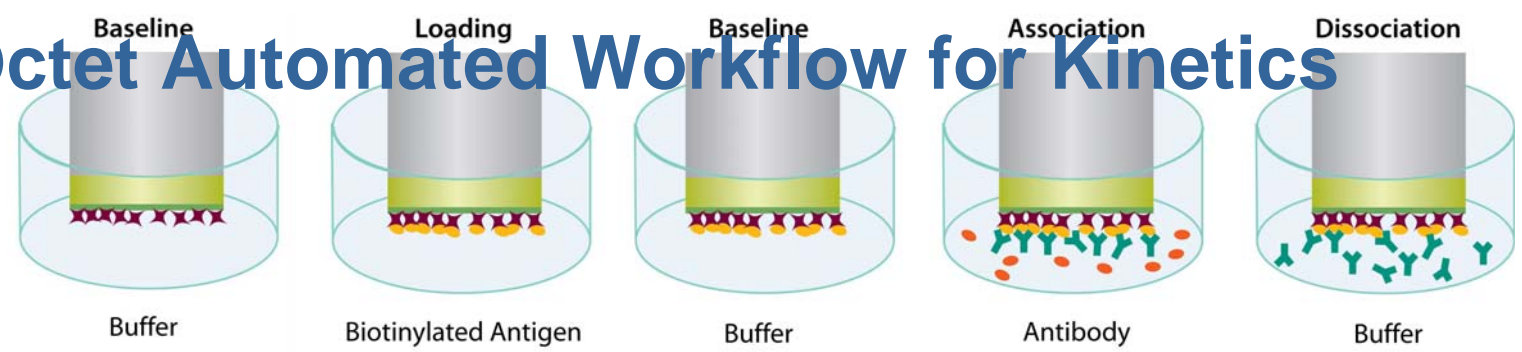
FortéBio Octet Technology

*forté*BIO™

Octet Technology

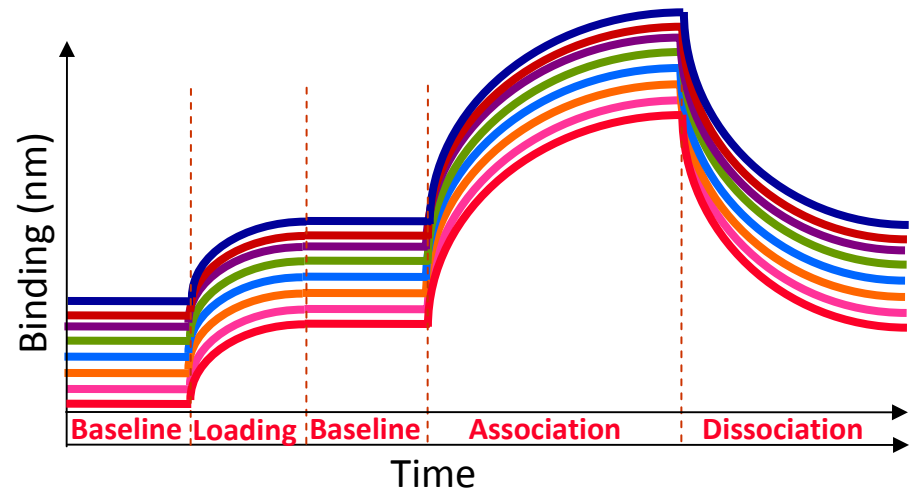
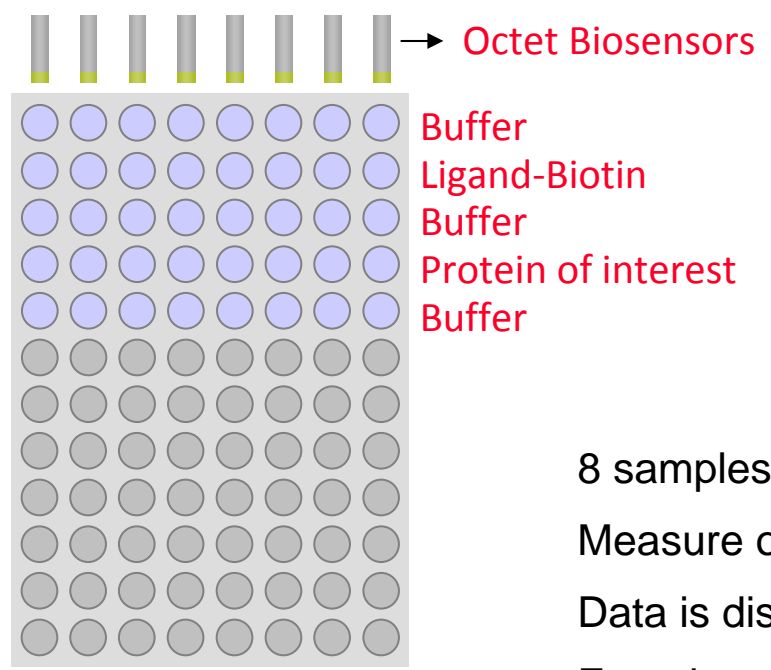


Octet Automated Workflow for Kinetics



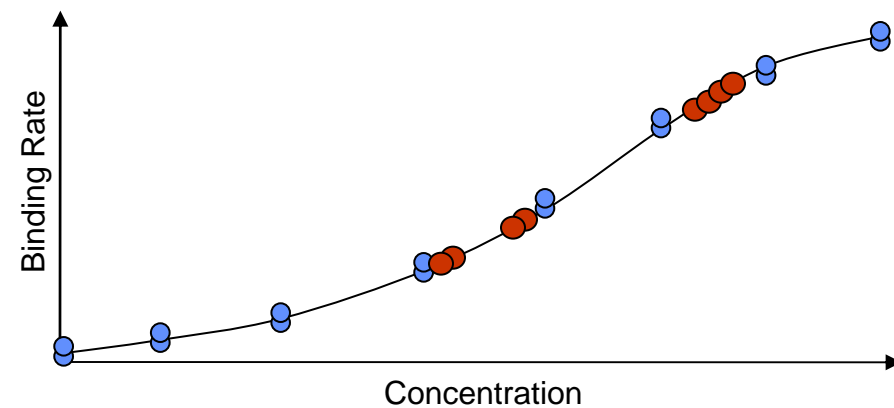
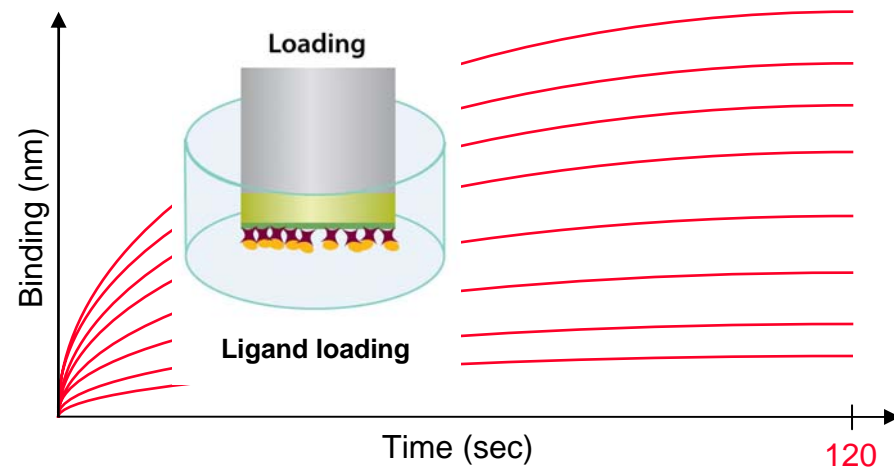
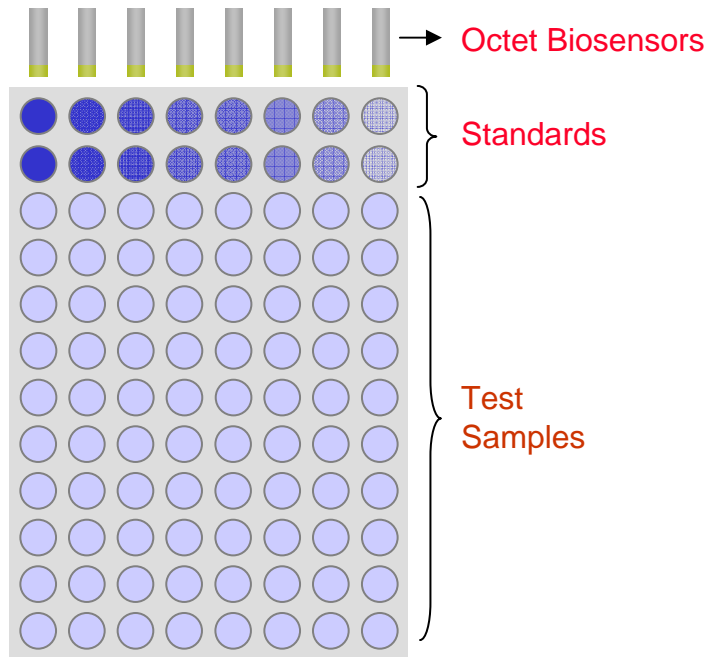
Legend

- Biosensor Tip
- Streptavidin
- Antigen
- Antibody
- Non-specific proteins



- 8 samples can be analyzed in parallel
- Measure on rates and off rates
- Data is displayed in real-time
- Experimental protocols can be customized

Octet Workflow for Quantitation



- Binding rates of standards are plotted vs conc.
- The binding rates of test samples are then measured and interpolated from the standard curve to determine their concentration
- 96 samples analyzed in 30 minutes

Titer method comparison

Methods compared for a 96-well plate or the equivalent # of samples

	ELISA	FortéBio	Prot A HPLC
Total time	6-8hrs	1.5-2hrs	8-10hrs
Hands on time	2-4hrs	1hr	2-4hrs
Sample consumed	2-10 μ l*	2-50 μ l**	10-90 μ l***
Linear range	0.031-0.5 μ g/ml	0.5-1000 μ g/ml	30-7000 μ g/ml (3-70 μ g injected)
Cost per "plate"	\$300-600	~\$300	~\$250
Automating data output	good	ideal	poor
Precision	5-20%	5-10%	5-10%

* Sensitivity of assay dictates samples being diluted

** 200ml loaded, sample dilution a minimum of 4-fold

*** Typical injection volumes, samples are rarely diluted



Octet assay evaluation

- Matrix effect
- Different antibodies
- Comparison to Prot A HPLC
- Comparison to ELISA
- Plate stability
- Plate to plate performance
- Sensor regeneration

Matrix effect



IgG spiked into different media at 5mg/ml

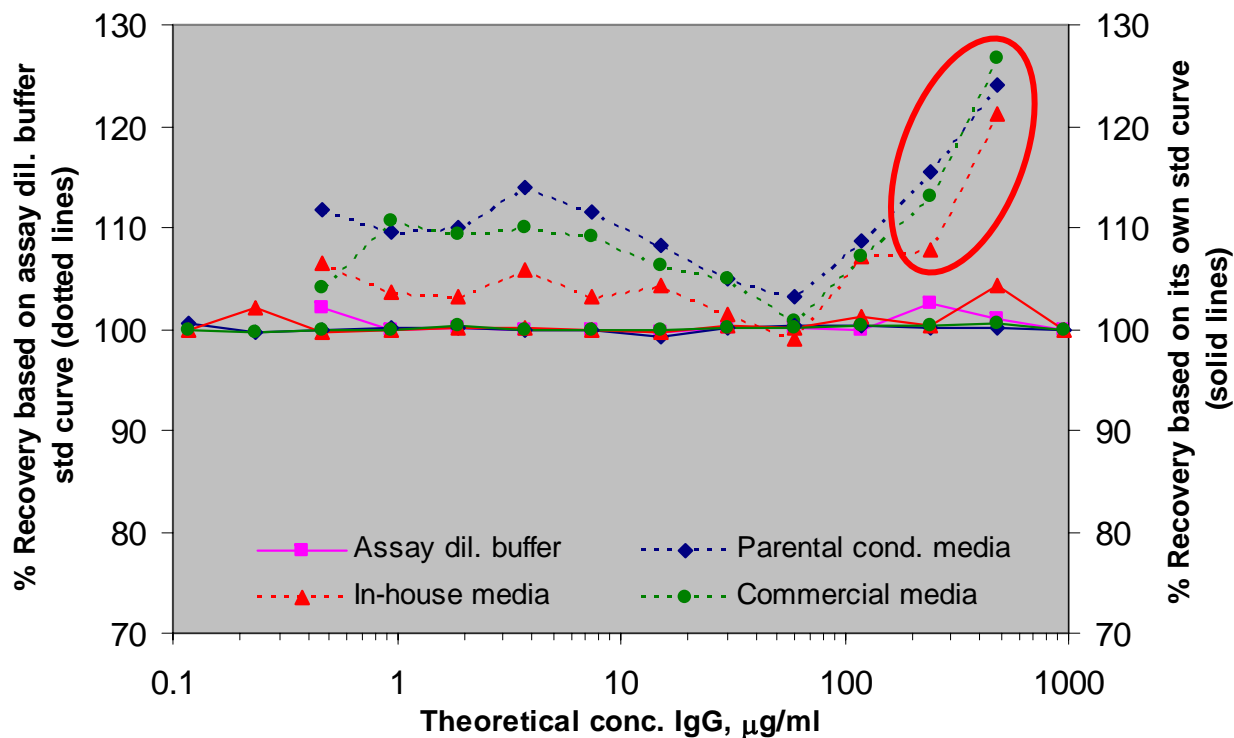


Different media samples diluted to 1000µg/ml in assay dilution buffer



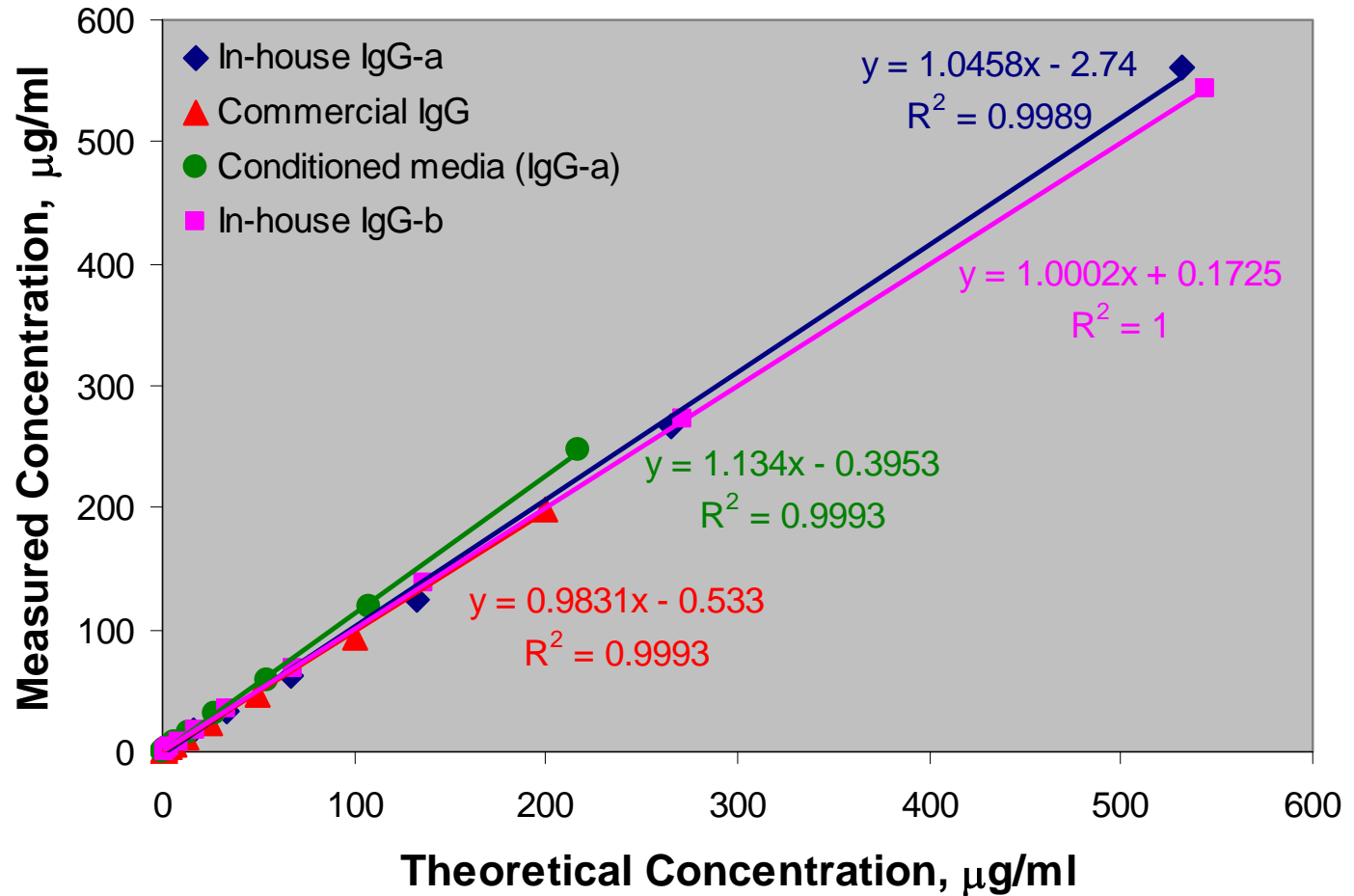
2-fold dilution series in assay dilution buffer

➔ 10-fold dilution required



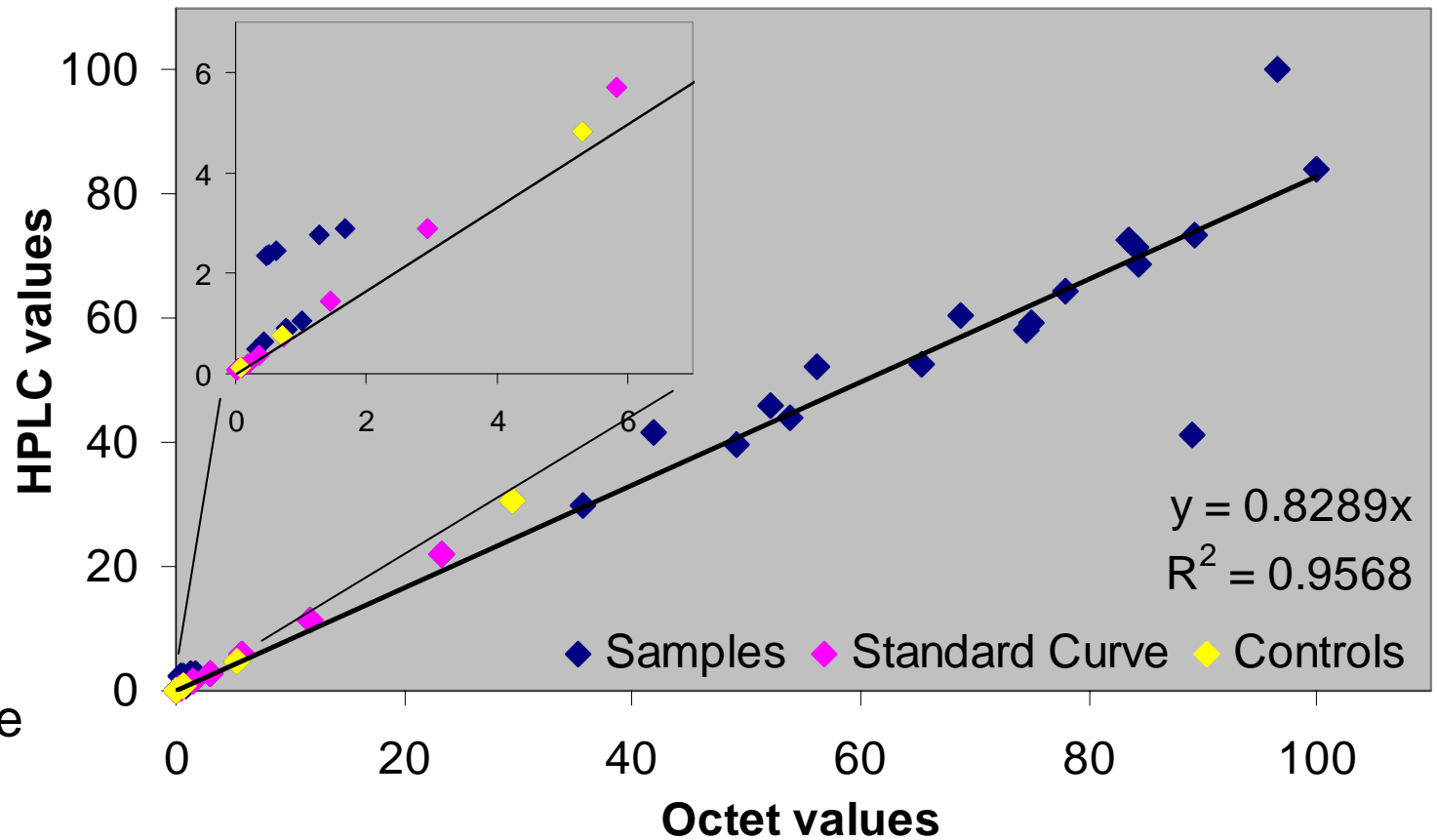
Different antibodies

Standard curves



Octet vs HPLC

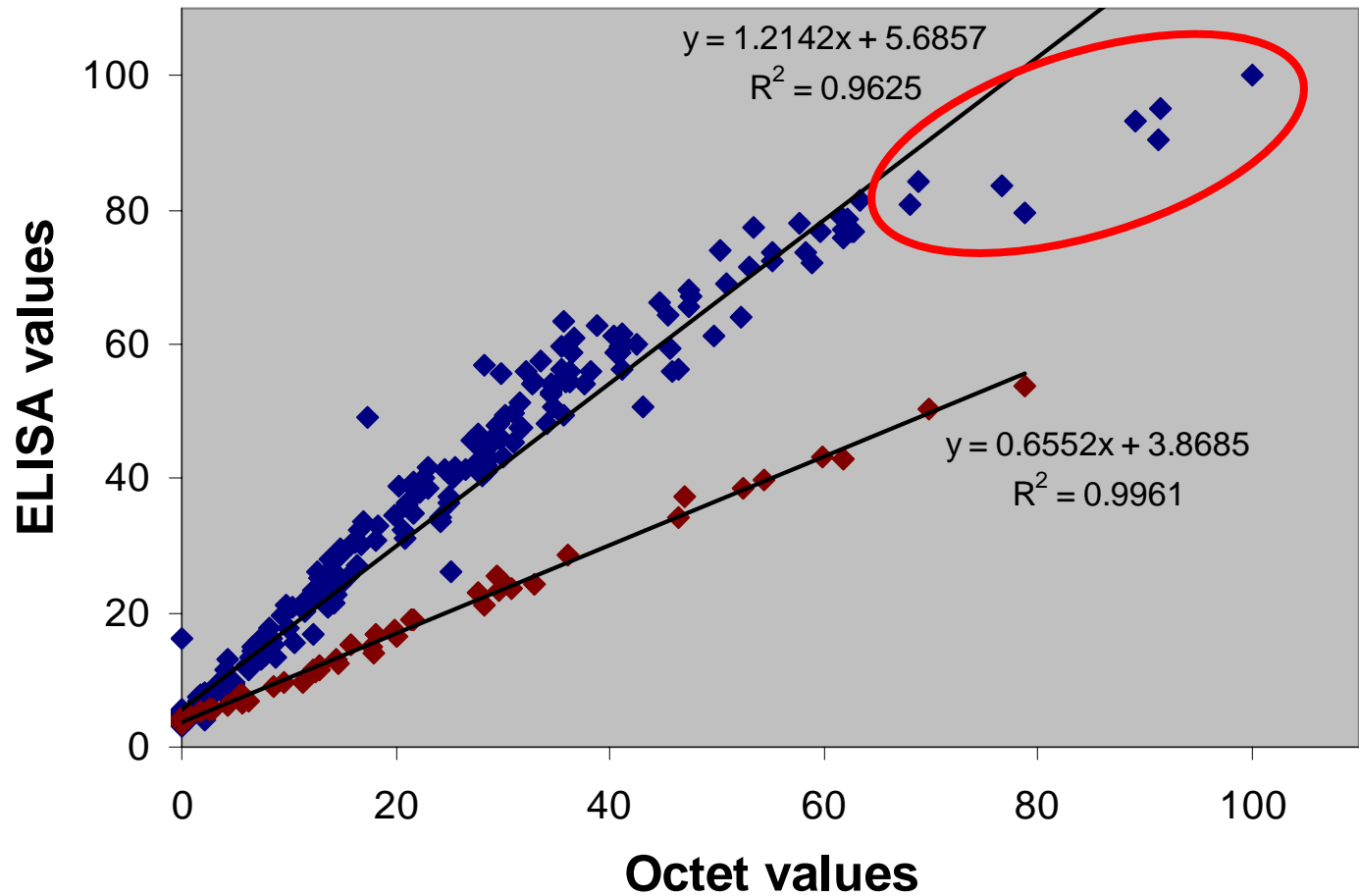
All values normalized to 100



30 samples
Standard curve
Controls

Octet vs ELISA

All values normalized to 100



5 plates (96-well)
Positive controls
Screening mode

Plate to plate comparison

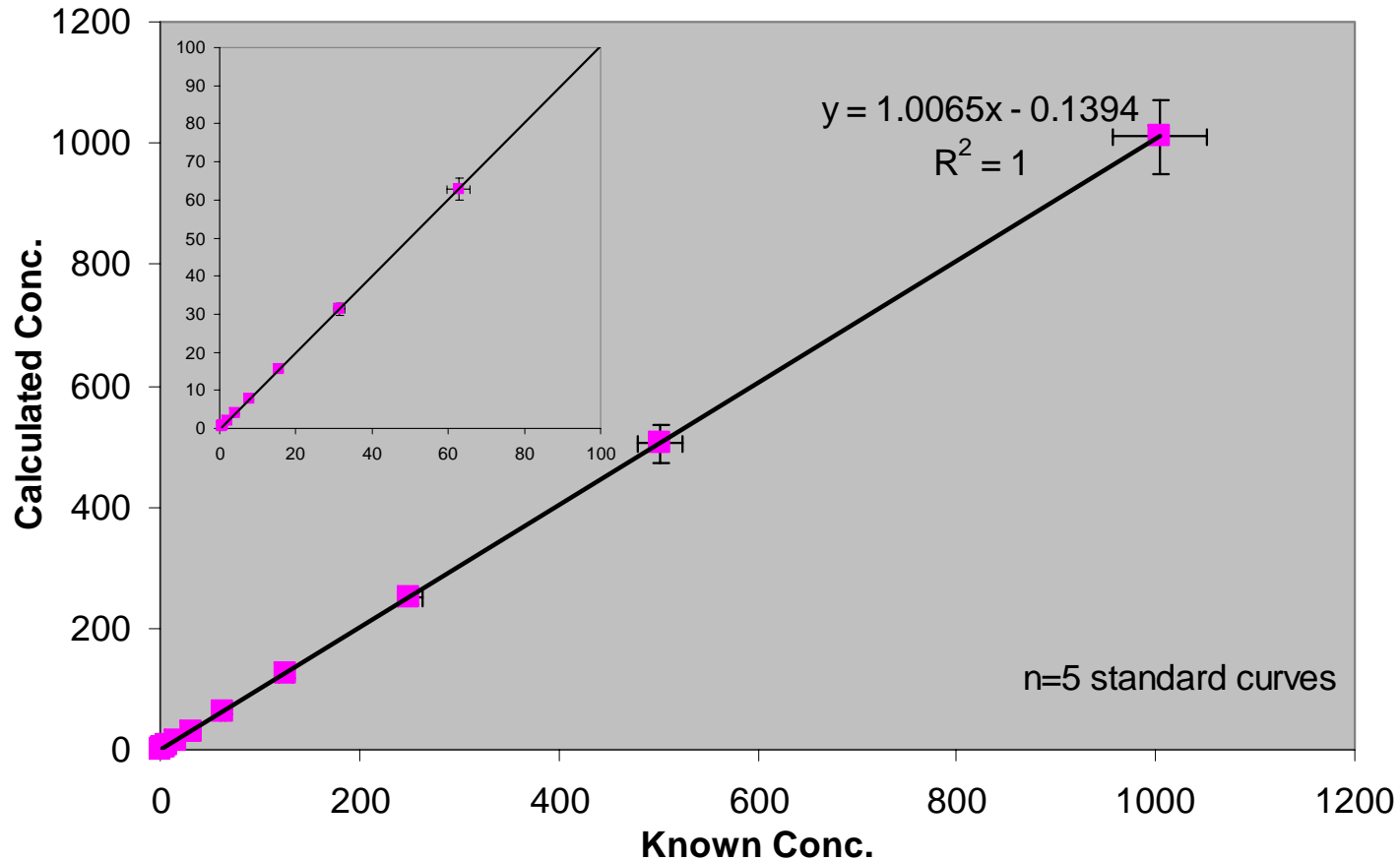
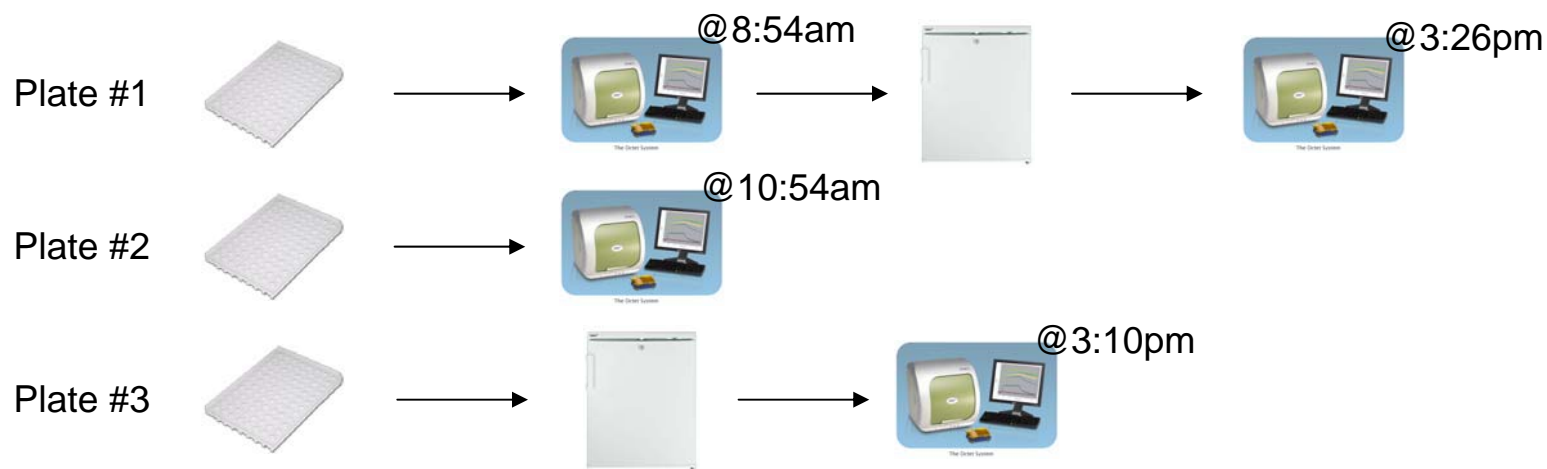


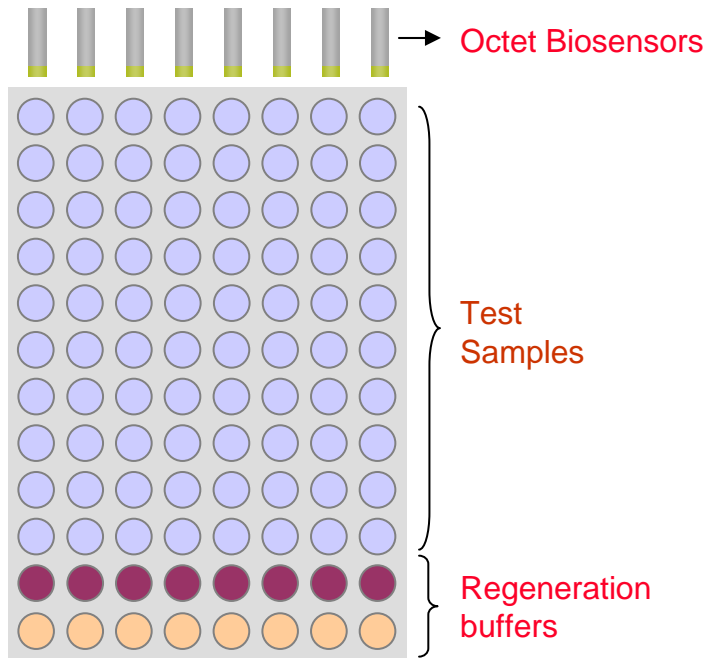
Plate stability



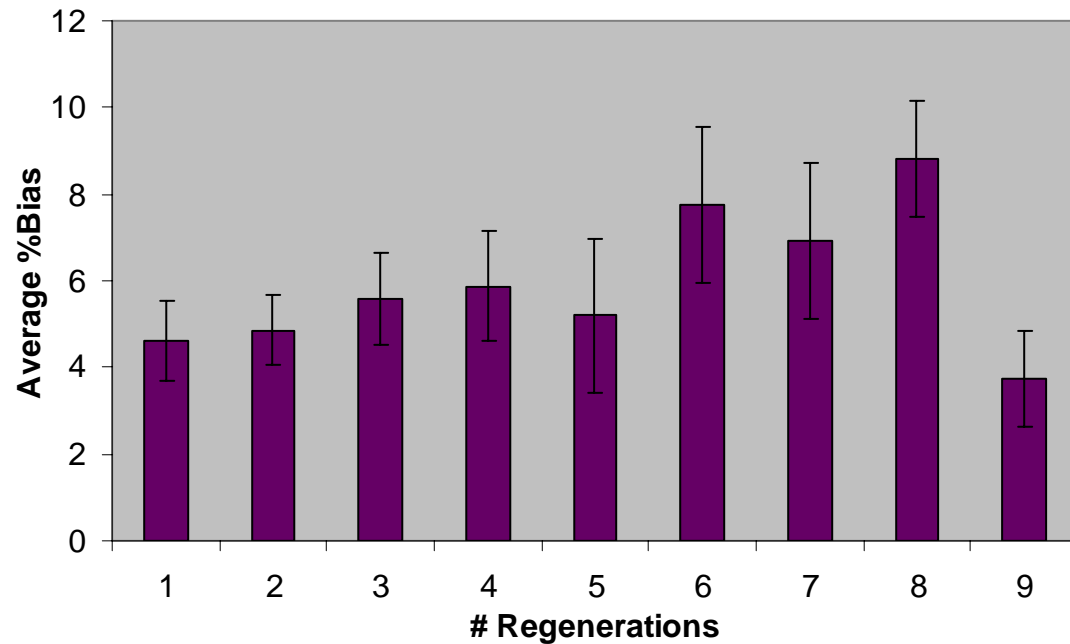
Time elapsed	IgG Conc. $\mu\text{g/ml}$								Plate #	$^{\circ}\text{C}$
	996.00	498.00	249.00	124.50	62.25	31.13	15.56	7.78		
--	996.00	498.00	249.00	124.50	62.25	31.13	15.56	7.78	1	RT
2hr	1000.00	463.00	244.00	119.00	61.60	30.00	15.10	7.42	2	RT
6hr 16min	1020.00	484.00	240.00	119.00	59.40	30.30	14.90	7.42	3	2-8 $^{\circ}\text{C}^*$
6hr 32 min	1050.00	556.00	236.00	129.00	60.90	31.00	15.20	7.55	1	2-8 $^{\circ}\text{C}^*$ after first read
mean	1016.50	500.25	242.25	122.88	61.04	30.61	15.19	7.54		
stdev	24.68	39.85	5.56	4.84	1.22	0.54	0.28	0.17		
CV%	2.4	8.0	2.3	3.9	2.0	1.8	1.8	2.3		
Bias%	-2.1	-0.5	2.7	1.3	1.9	1.7	2.4	3.1		

* Plate was allowed to warm to room temperature for 20 min before reading

Prot A Sensor regeneration



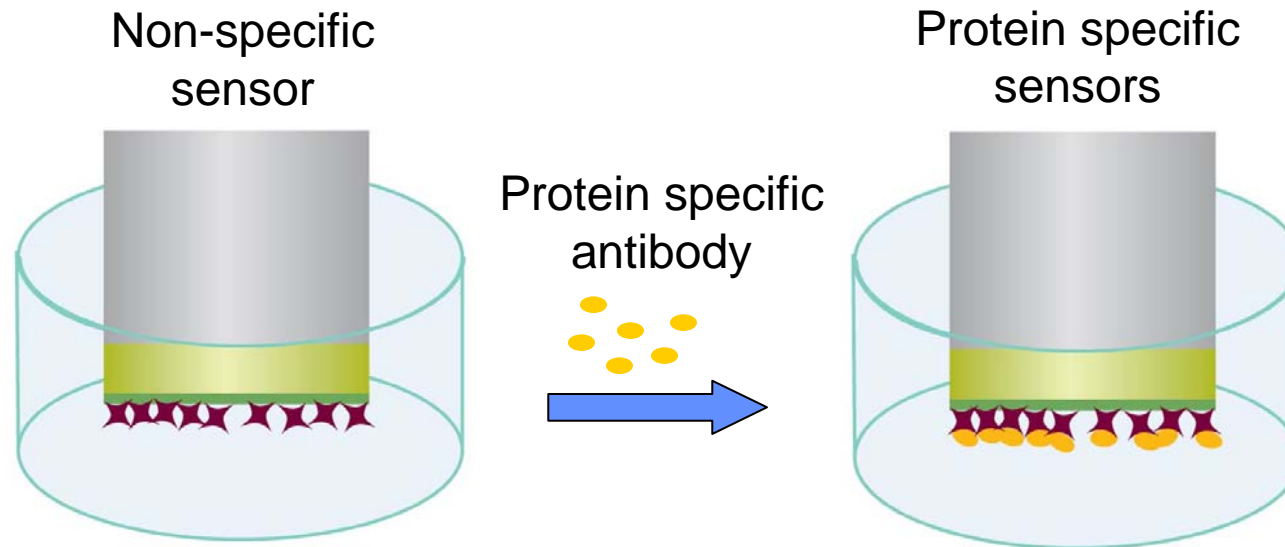
Prot. A sensors can be regenerated
Between each reading – cycle through
stripping buffer and wash
Sensor reused up to 5 times



Assay development for non-IgG's

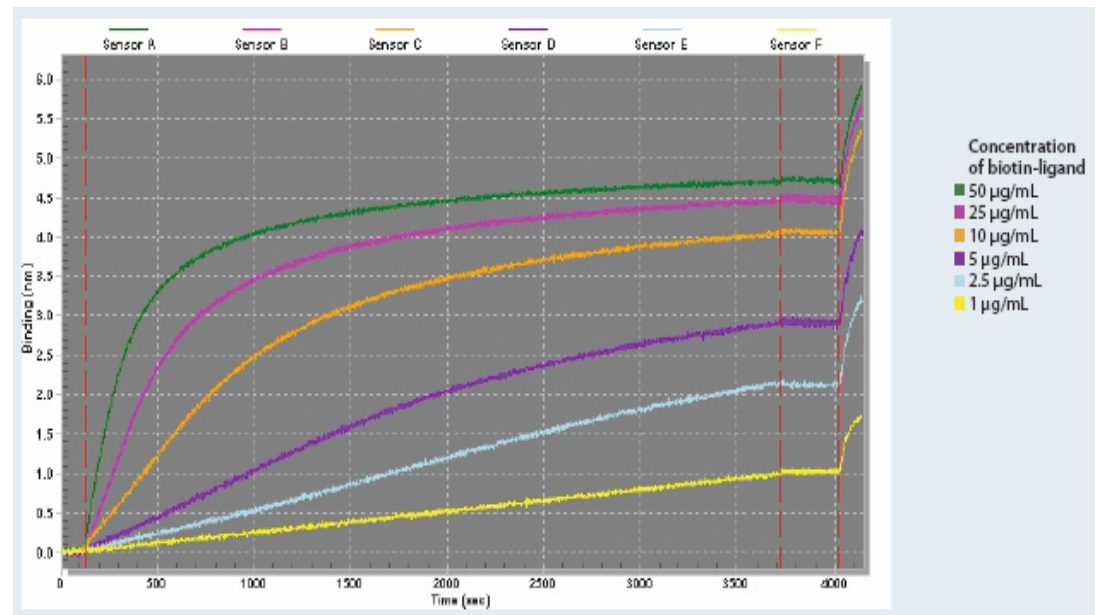
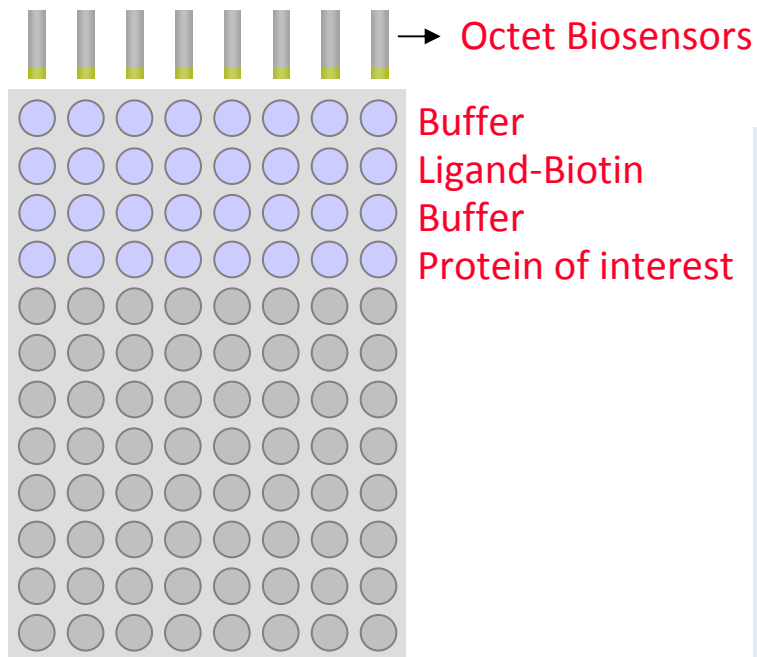
Build your own custom protein specific sensors:

- Streptavidin sensors – biotinylated antibody
- Anti-human IgG sensors – human antibody
- Anti-mouse IgG sensors – murine antibody



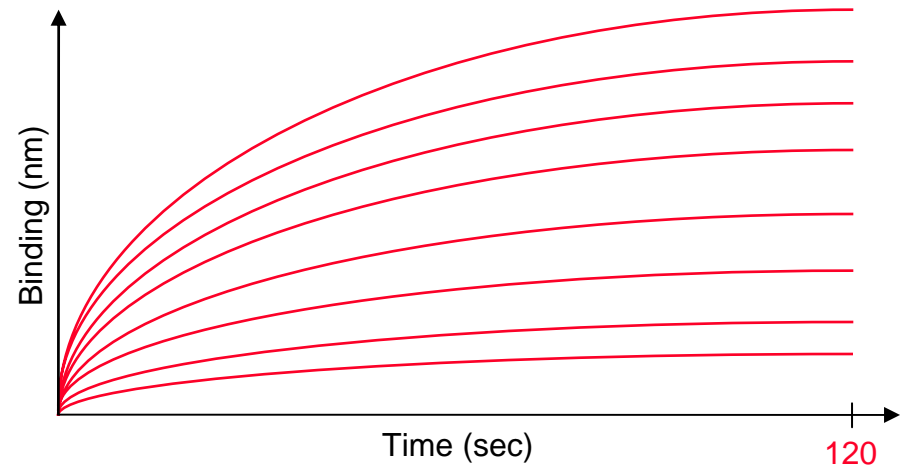
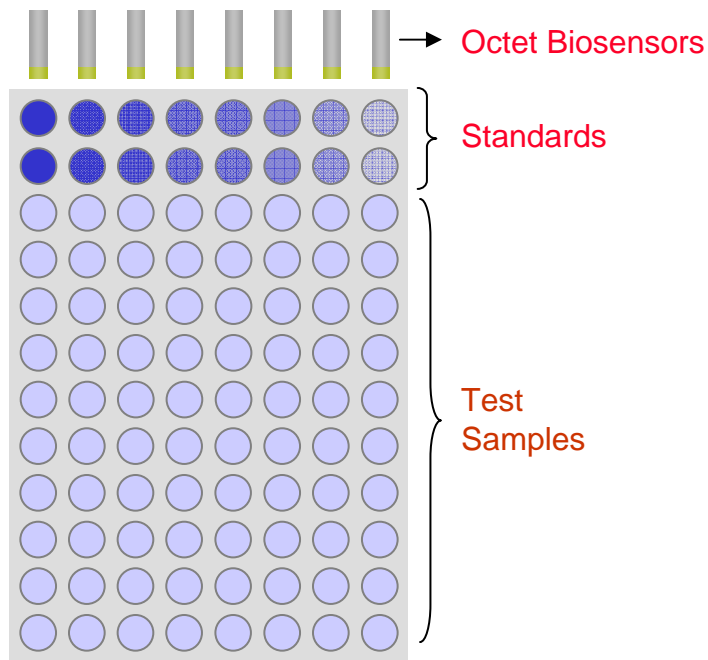
Assay development for non-IgG's

1) Optimize the immobilization of the ligand onto the sensors

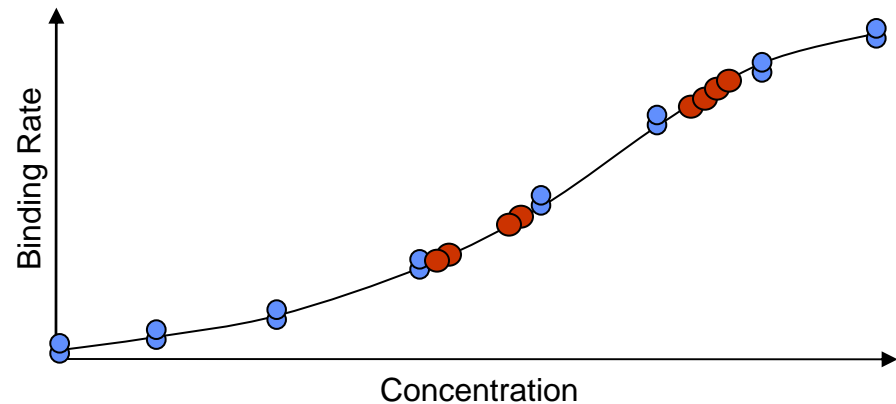


2) Optimize immobilization conditions for off-line

Assay development for non-IgG's



- 3) Optimize the assay parameters, dynamic range, and precision
- 4) Preserve coated biosensors for storage and assess stability



Summary

- Improved throughput over ELISA and Prot A HPLC
- Linear range similar to Prot A HPLC: 0.875 - 957µg/ml
- Good correlation between assays
- Sensor regeneration software provide significant cost savings for quantitation assays
- Versatile, assays for non-IgG's can be developed

Acknowledgements

