

## Case Study 2

# Ultrafast, Single B Cell-based Anti-idiotypic mAb Discovery Against a mAb Therapeutic

Delivering PK assay-ready mAbs in 11 weeks to meet aggressive clinical timelines

## Applications and Background

- Development of assay-ready mAbs for a PK assay for a therapeutic antibody (IgG) going into trials very soon
- Partner failed a discovery campaign internally (poor titer) and brought in Abveris help to avoid delaying clinical trials



Urgent delivery required  
(< 3 months)

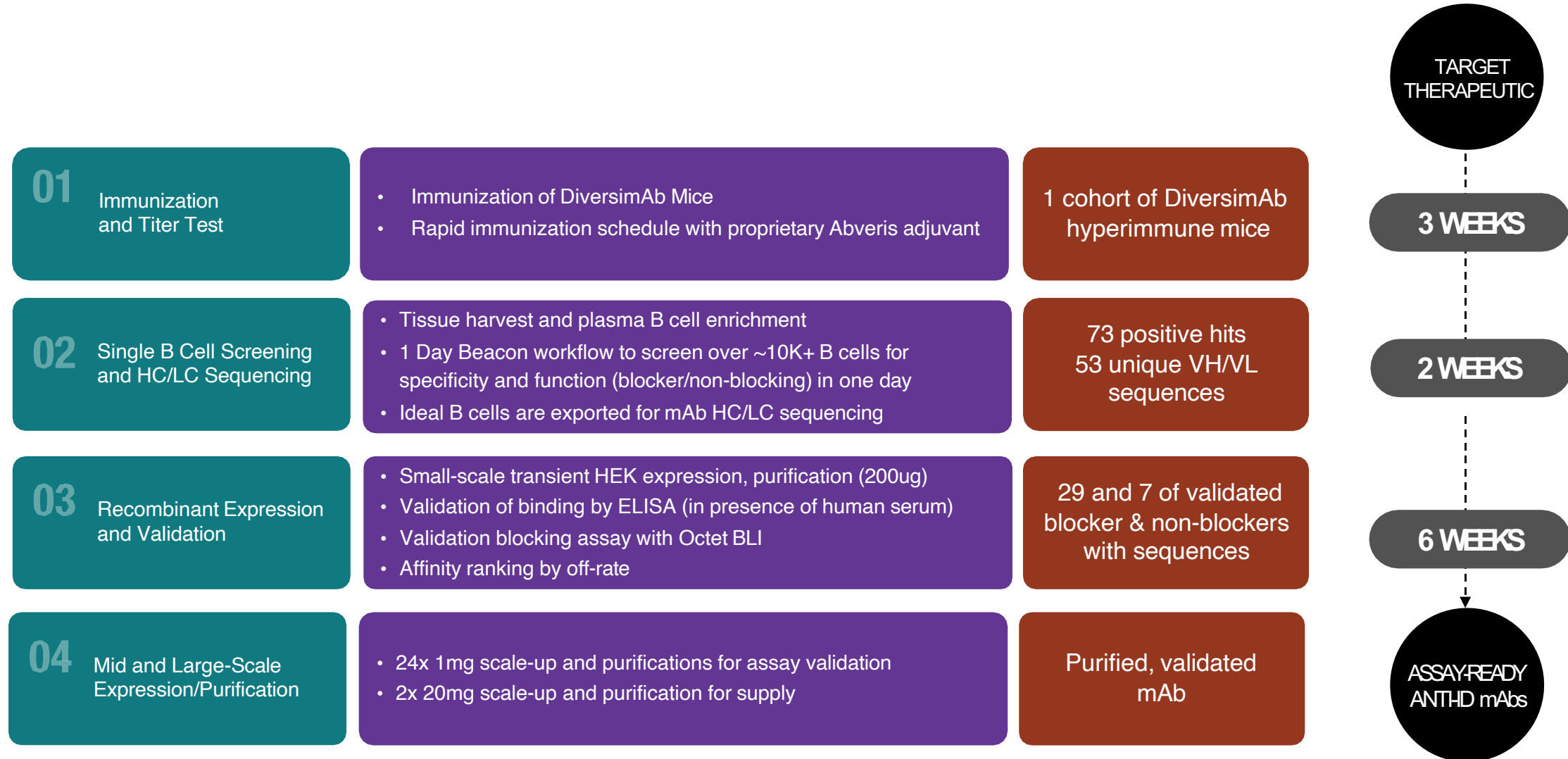


## Requirement and Requested Deliverables

- High specificity, no off-target binding for clean PK assay results
- Blocking and non-blocking mAbs required
- A range of affinities
- Antibody sequences and validated small-scale, purified antibodies now. Larger quantities of mAb soon after.
- ASAP timelines (less than 3 months)

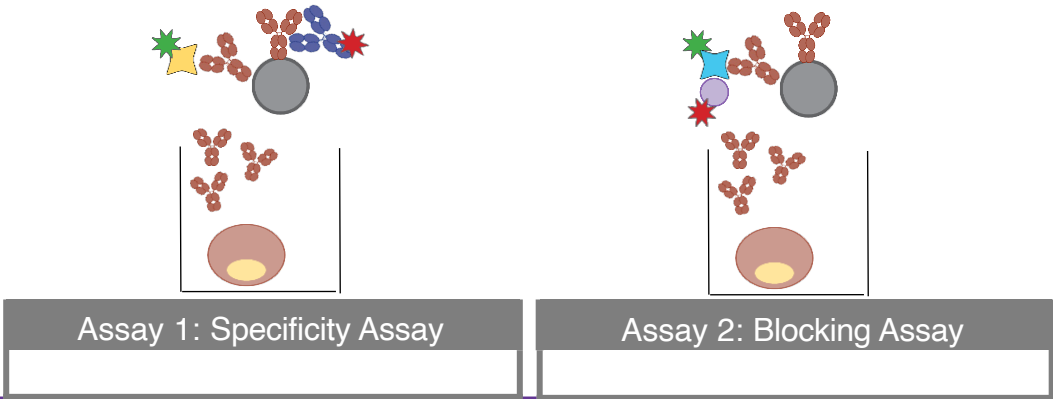
# ANTI-IDIOTYPIC ANTIBODIES DISCOVERY- ULTRAFAST, SINGLE B CELL WORKFLOW

Delivering PK assay-ready mAbs in 11 weeks to meet aggressive clinical timelines



# BEACON-BASED SINGLE CELL ASSAYS FOR BINDING PROFILE

Multiplex, sequential assays for (1) specificity to target Fab, with no reactivity to (-) control antibody and (2) ligand-blocking assay



	AF647	AF488	AF647	AF488
Target-specific binder, Ligand non-blocker	+	-	+	+
Target-specific binder, Ligand blocker	+	-	-	+
Target-specific binder, partial-blocker (or low affinity binder)	+	-	-	-
Nonspecific binder	+	+	+ or -	+ or -
Non-binder	+	-	-	-

IgG excreting B cells  
screened = 10,784

Hit Count

50

23

Legend

Live plasma B cell

B cell secreted Ab

Goat anti-mouse Fc-AF647 (detection Ab)

Goat anti-Mouse Fc beads

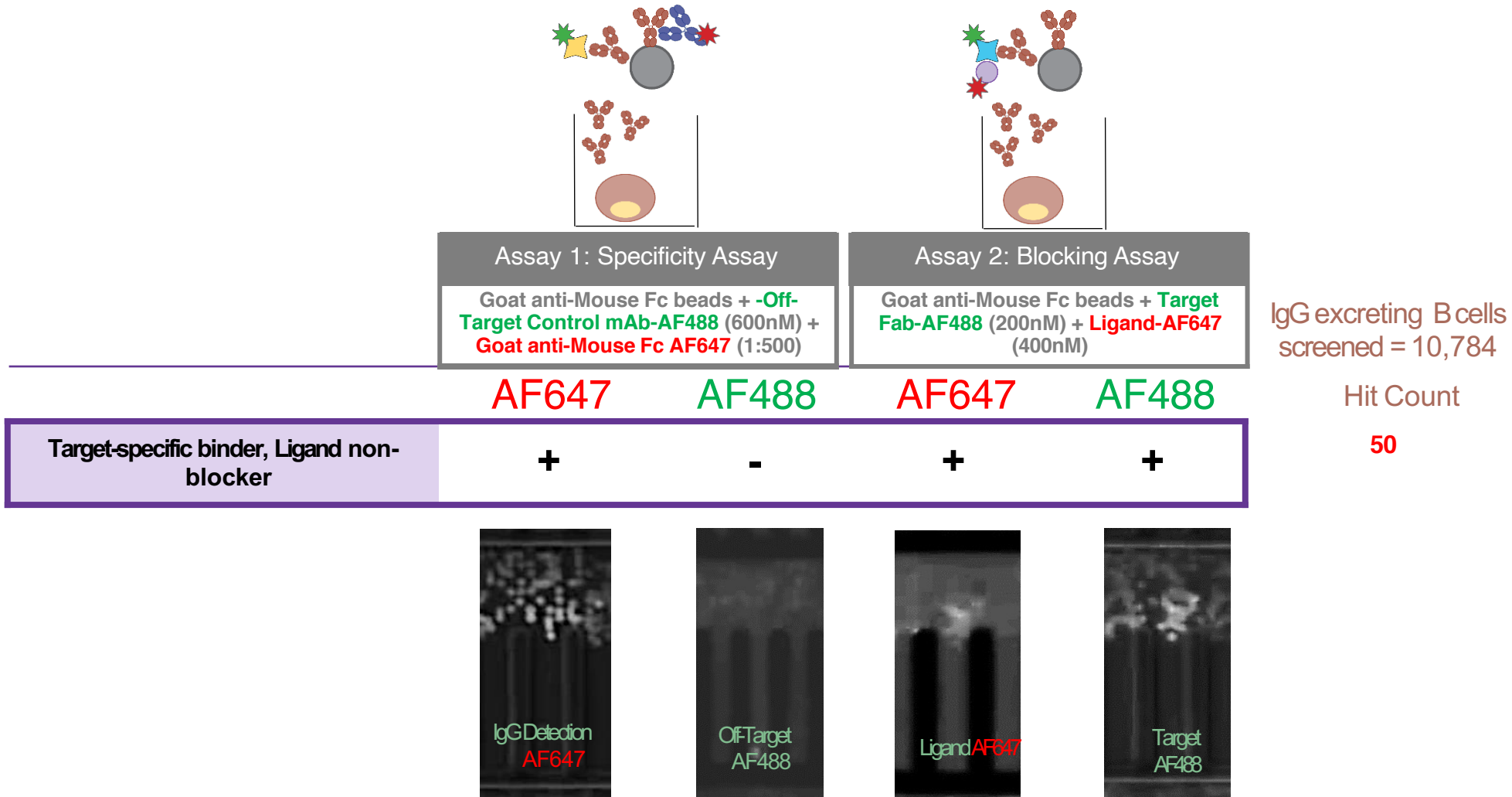
Off-target Control

Target Fab-AF488

Ligand-AF647

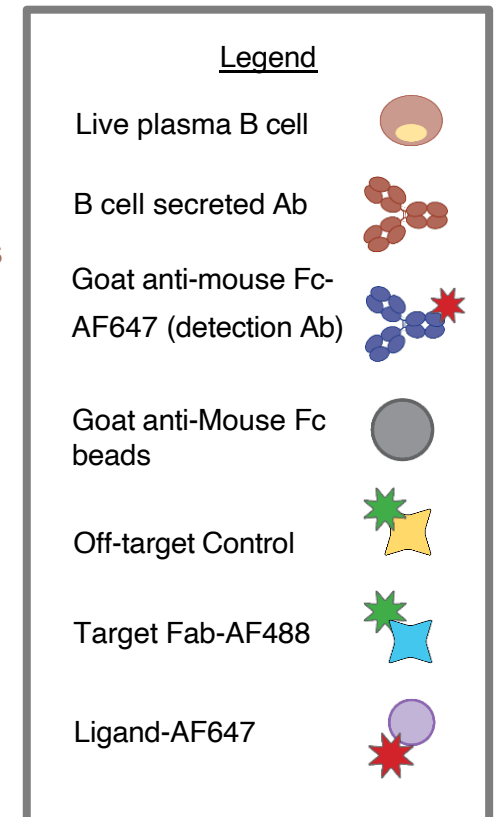
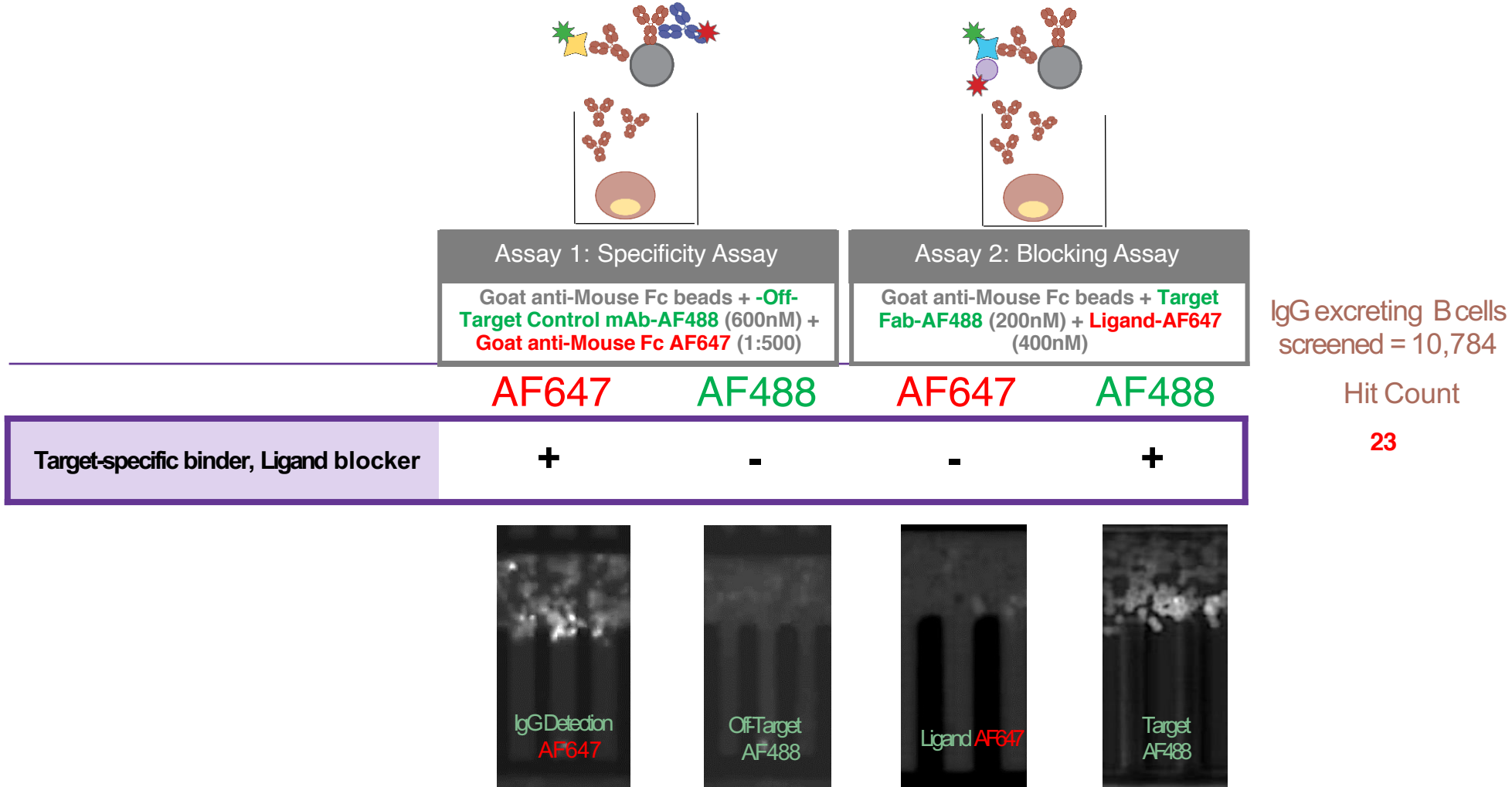
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# ANTIBODY EXPRESSION AND DOWNSTREAM VALIDATION

Antibodies were expressed and further validated by ELISA and Octet for binding profile

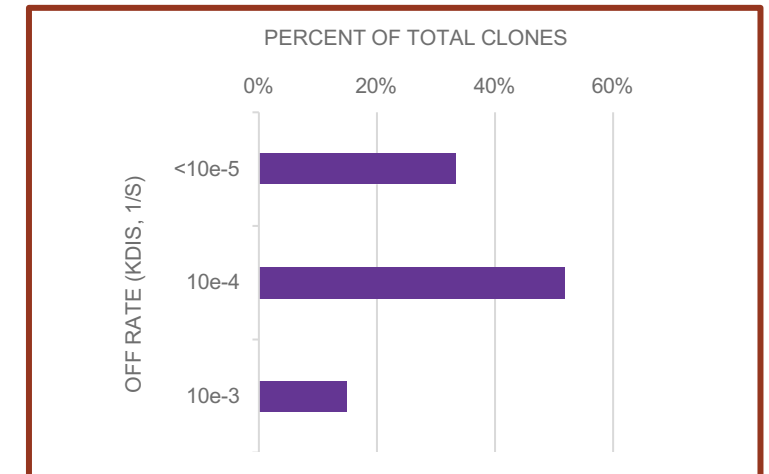
Number of Antibodies	1 Unique sequences obtained	2 Small scale expression	3 ELISA validated	4 Blocking assay by Octet BLI
	37	33	36	29
Blocker	37	33	36	29
Non-Blocker	16	14		7

89% recovery

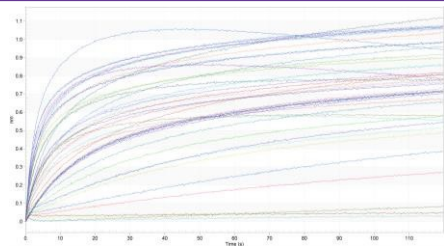
77% recovery

100% recovery

Affinity Ranking of 36 Octet BLI validated antibodies



## 3 Small-Scale Expression



Recombinant expression  
89% efficiency by Octet validation  
(confirmed expression)

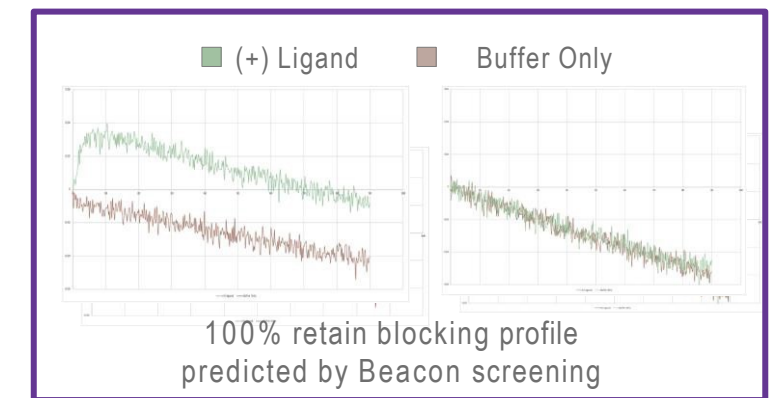
Small-scale high throughput Expi293  
expression and purification:  
Average 225  $\mu\text{g} \pm 60 \mu\text{g}$  yield

## 4 ELISA Validation in the Presence of Human Serum

Clone	Target Fab (+)	Target Fab in 20% NHS (+)	Target IgG (+)	Irrelevant Fab-His (-)	Poly Hu IgG (-)
1	1.0868	0.824	1.0647	0.0572	0.0598
2	1.0036	0.3936	1.0998	0.0715	0.13
3	0.9802	0.4544	0.9594	0.0585	0.0624
4	0.9243	0.3792	0.8541	0.0559	0.0585
5	0.8879	0.1776	0.9711	0.0572	0.0663
6	0.8762	0.2576	0.9425	0.0559	0.0598
7	0.6188	0.144	0.6279	0.0598	0.3588

ELISA screening cascade including matrix using 20% human serum, 77% retain activity in the presence of human serum  
7 example clones shown

## 5 Octet Blocking Assay for activity validation



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## Conclusions

- 11 weeks from the start of immunization to delivery of validated small-scale, purified antibodies with sequences
- High-quality mAbs delivered – specific, high-affinity mAbs with blocking and non-blocking profiles
- Partner was able to develop highly sensitive PK assay and move clinical trials forward on time