Case Study 2

Ultrafast Single B Cell-based Anti-idiotypic mAb Discovery against an mAb Therapeutics

RAPID, B CELL-BASED ANTI-IDIOTYPIC ANTIBODY DISCOVERY

Abveris
A Division of Twist Bioscience

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

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

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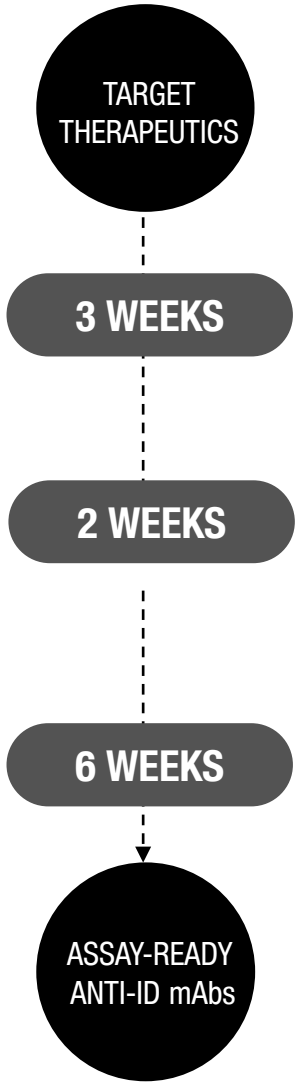
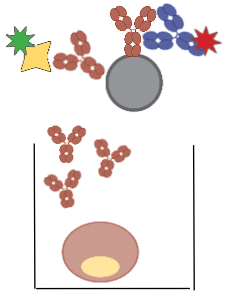
01	Immunization and Titer Test	• Immunization of DiversimAb Mice • Rapid immunization schedule with proprietary Abveris adjuvant	1 cohort of DiversimAb hyperimmine mice
02	Single B Cell Screening and HC/LC Sequencing	• Tissue harvest and plasma B cell enrichment • 1 Day Beacon workflow to screen over ~10K+ B cells for specificity and function (blocker/non-blocking) in one day • Ideal B cells are exported for mAb HC/LC sequencing	73 positive hits 53 unique VH/VL sequences
03	Recombinant Expression and validation	• Small-scale transient HEK expression, purification (200ug) • Validation of binding by ELISA (in presence of human serum) • Validation blocking assay with Octet BLI • Affinity ranking by off-rate	29 and 7 of validated blocker & non-blockers with sequences
04	Mid and Large-Scale Expression/Purification	• 24x 1mg scale-up and purifications for assay validation • 2x 20mg scale-up and purification for supply	Purified, validated mAb

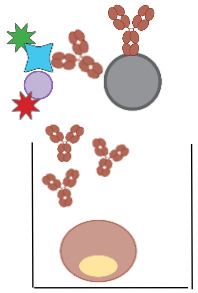


TWIST BIOSCIENCE

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





	Assay 1: Specificity Assay Goat anti-Mouse Fc beads + -Off-Target Control mAb-AF488 (600nM) + Goat anti-Mouse Fc AF647 (1:500)		Assay 2: Blocking Assay Goat anti-Mouse Fc beads + Target Fab-AF488 (200nM) + Ligand-AF647 (400nM)		IgG excreting B cells screened = 10,784 Hit Count
	AF647	AF488	AF647	AF488	
Target-specific binder, Ligand non-blocker	+	-	+	+	**50**
Target-specific binder, Ligand blocker	+	-	-	+	**23**
Target-specific binder, partial-blocker (or low affinity binder)	+	-	-	-	
Nonspecific binder	+	+	+ or -	+ or -	
Non-binder	+	-	-	-	

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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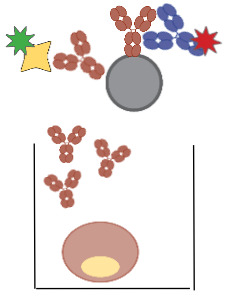

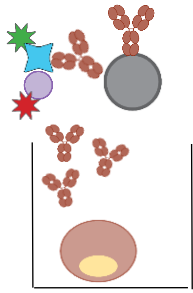

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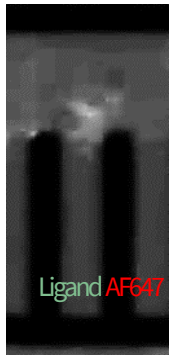
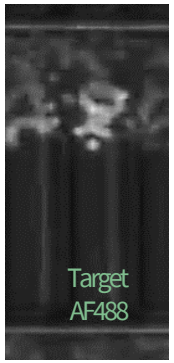
	AF647	AF488	AF647	AF488
Target-specific binder, Ligand non-blocker	+	-	+	+

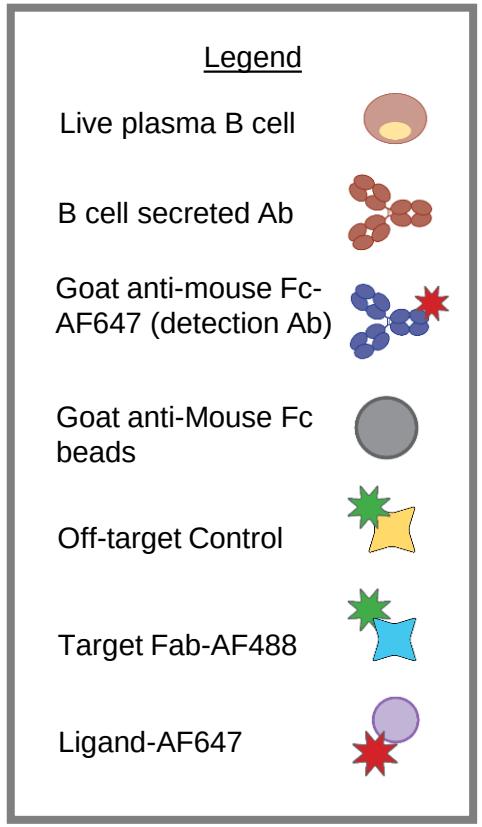
IgG excreting B cells screened = 10,784

Hit Count

50







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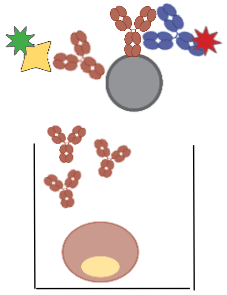

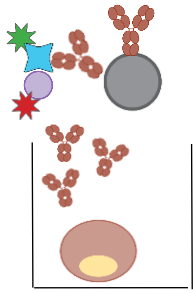

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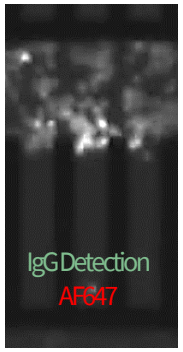

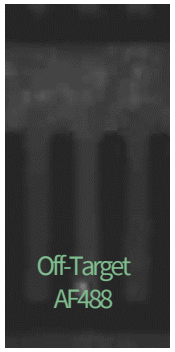

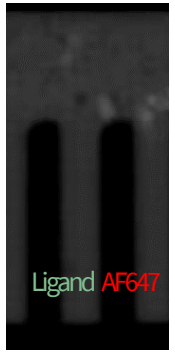

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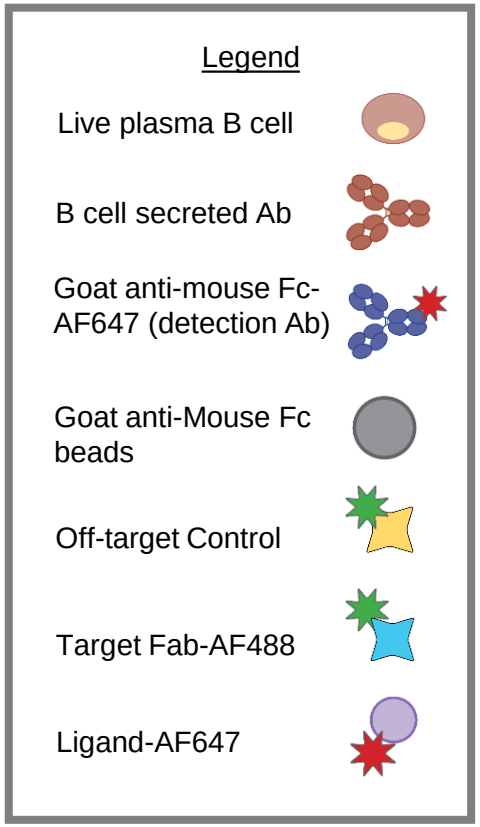


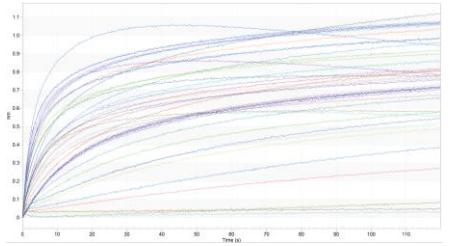
ANTIBODY EXPRESSION AND DOWNSGREAM VALIDATION

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

73% recovery → 89% recovery → 77% recovery → 100% recovery

Number of Antibodies	1 Hits exported for sequencing	2 Unique sequences obtained	3 Small scale expression	4 ELISA validated	5 Blocking assay by Octet BLI
Blocker	**50**	**37**	**33**	**36**	**29**
Non-Blocker	**23**	**16**	**14**		**7**

3 *Small-Scale Expression*



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

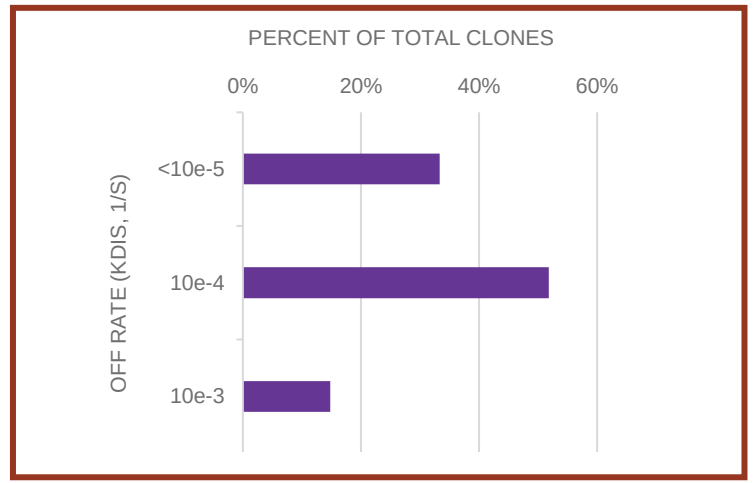
Small-scale high throughput Expi293 expression and purification:
Average 225 µg ± 60 µg yield

4 *ELISA Validation in the Presence of Human Serum*

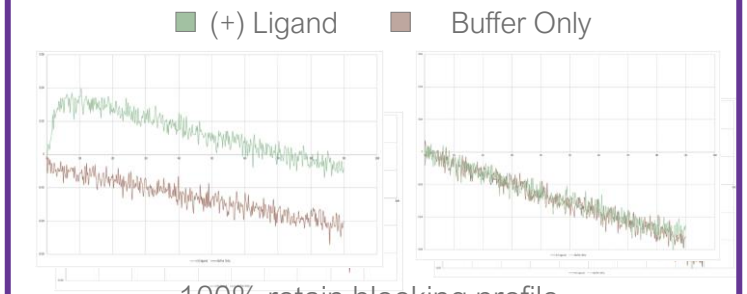
Clone	Target Fab (+)	Target Fab in 20% NHS (+)	Target IgG (+)	Irrelevant Fab-His (-)	Poly HulgG (-)
1	1.0868	0.824	1.0647	0.0572	0.0598
2	1.0036	0.3936	1.0998	0.0715	0.13
3	0.9802	0.4544	0.9594	0.0585	0.0624
4	0.9243	0.3792	0.8541	0.0559	0.0585
5	0.8879	0.1776	0.9711	0.0572	0.0663
6	0.8762	0.2576	0.9425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

Affinity Ranking of 36 Octet BLI validated antibodies



5 *Octet Blocking Assay for activity validation*



100% retain blocking profile
predicted by Beacon screening

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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