Antibody Specificity Measurement by Exploration of Diverse Sequence Space

Merck KGaA
Darmstadt, Germany

Authors:
Simon L. Goodman Ph.D., Principal Scientist, Merck KGaA, Darmstadt Germany
Matthew Greving, Ph.D.; Jon Melnick, Ph.D.; Theodore M. Tarasow, Ph.D. HealthTell Inc., San Ramon, CA & Chandler, AZ

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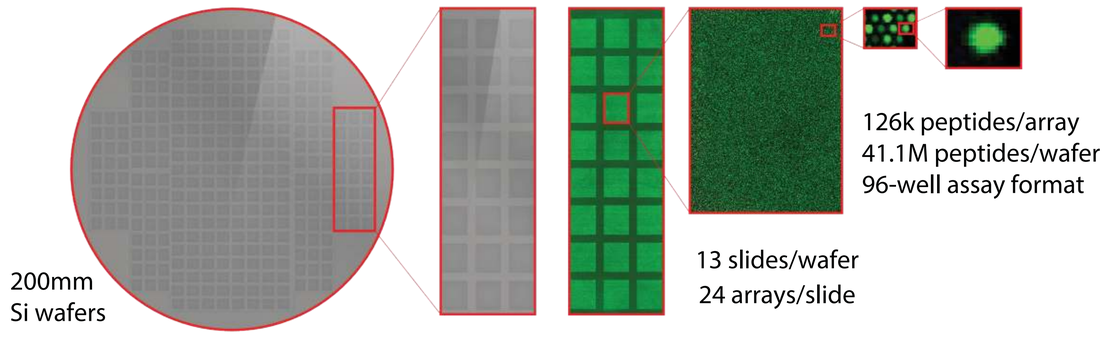
Abstract

Antibody binding specificity is a major concern in research, therapeutic antibody development and diagnostics. The wide-range of antibody binding specificity between clones, vendors, lots, hosts, formats, etc. is well documented. Protein arrays, Western Blots, immunohistochemical stains and small peptide libraries are typical approaches in assessing antibody specificity. These specificity measurement approaches have significant limitations including: narrow sequence space coverage, limited throughput, difficulty in identifying binding target(s) and a lack of quantitative capability. A need exists for quantitative, information-rich antibody specificity measures to improve throughput and quality of mAb candidate selection.

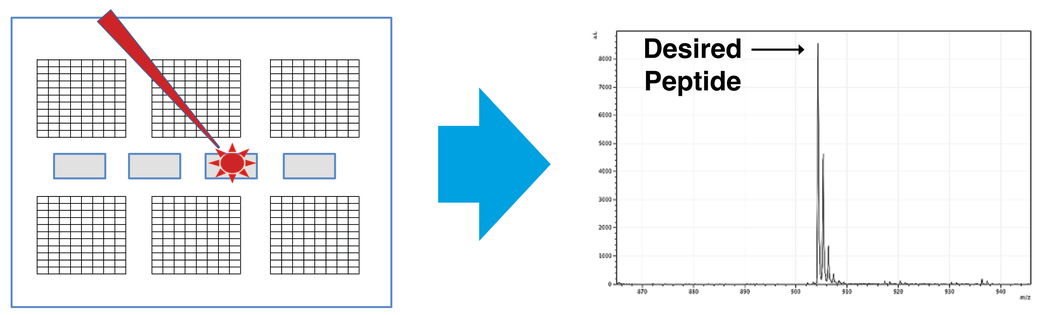
Merck KGaA identified HealthTell's high-density peptide array as a platform to address the need for a quantitative antibody binding specificity measurement, without the limitations of existing approaches. Specifically, on the HealthTell Platform: 1) Assays are performed in a high-throughput 96-well format, 2) Diverse specificity metrics are measured with a 126K peptide members array library in each well, 3) Peptide library binding profiles can be used to quantify specificity without prior antibody epitope knowledge, and 4) Peptide library information content is sufficient to map binding profiles to sets of antibody targets for antibody binding/cross-reactivity propensity ranking within that set.

HealthTell's High-Density Array Platform

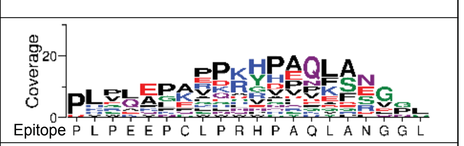
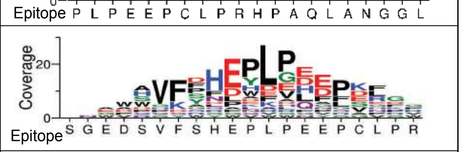
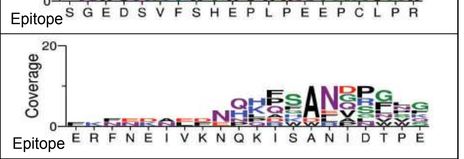
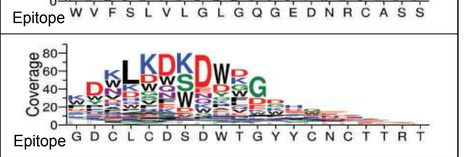
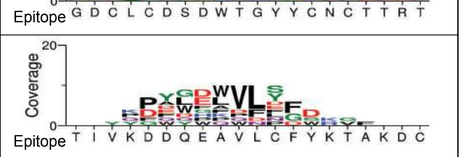
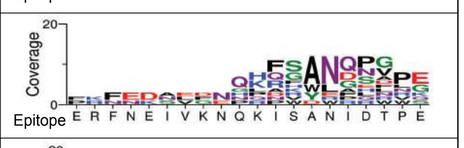
Scalable, Reproducible Photolithographic Synthesis of High-Quality Peptide Arrays



In Situ Array MALDI-MS Confirms Each Synthesis Cycle and Full Peptide Synthesis



Merck KGaA mAb Panel and Array Binding Consensus

mAb ID	mAb Target	Enriched Binding Motif(s)	Enrichment p-value(s)	Significant Peptides Conservation & Putative Epitope Coverage
5R	FGFR1	HPAQ PAQL	8.3E-09 2.5E-07	
6R	FGFR1	VFHEP HEPLP	1.1E-12 8.5E-07	
7R (12R Rep.)	ITGB6	FVND NDPVG	1.2E-17 1.1E-06	
8R (13R Rep.)	ITGB8	VLF GFELV	1.2E-40 7.3E-07	
9R	ITGB3	KDWS KDKD	3.8E-67 3.4E-30	
10R	ITGB5	WVPV VWWD	1.8E-12 4.2E-09	
12R (7R Rep.)	ITGB6	FVND NDPVG	1.7E-11 2.1E-08	
13R (8R Rep.)	ITGB8	VLF GFELV	2.3E-45 6.1E-06	

7R & 12R Reps 8R & 13R Reps

An initially blinded panel of mAbs, including blinded replicates (7R & 12R, 8R &13R), were provided to HealthTell by Merck KGaA for comprehensive binding characterization on HealthTell peptide arrays. Significant array binding peptides for each mAb produced enriched sequence motifs and sequence conservation that could be aligned to putative target epitopes. Array binding correctly identified all blinded replicates and two putative epitopes (5R & 6R, anti-FGFR1) have been confirmed by Merck KGaA.

Apparent mAb Specificity Rank

Increasing Specificity!

mAb ID	mAb Target	Specificity Assay Significant Peptide Count
6R	FGFR1	15
8R	ITGB8	18
7R	ITGB6	19
12R	ITGB6	19
13R	ITGB8	24
5R	FGFR1	29
10R	ITGB5	93
9R	ITGB3	94

7R & 12R Reps 8R & 13R Reps

7R Top Specific Peptides	12R Top Specific Peptides	8R Top Specific Peptides	13R Top Specific Peptides
FVQWRED	FVQWRED	ELLFRLDG	ELLFRLDG
KYSREDGRVLF	KYSREDGRVLF	RFLEVRFD	RFLEVRFD
YPVQLREG	YPVQLREG	FVELVFSG	PFLELVLS
NHPRNYRED	HVRWWNRED	FFSLVLEG	FFSLVLEG
WRLFVQDRED	WRLFVQDRED	FFEFSLFE	FFEFSLFE
YFPKWQWRED	YFPKWQWRED	VVFEFRLD	VVFEFRLD
LVNFRED	LVNFRED	GVFFRFEG	YSFLEKVFD
ARDYDGNPFSG	ARDYDGNPFSG	LAVELSLSE	LAVGFEVLS
YVQDPVGDG	YVQDPVGDG	YLRALFELE	LYLAFNLED
PPPQLRDG	LLLNVSDG	AEQRFPQYALG	AEQRFPQYALG

1) A mAb concentration series was bound to the peptide array in the presence of increasing spiked competitor. 2) Array feature-level binding dose response curves were generated for each mAb in the presence & absence of spiked competitor. 3) Significant peptides are defined as those with < 10-fold reduction in dose response with competitor vs. no competitor. 4) Total significant peptide counts represent a measure of apparent mAb binding specificity.
8R ~ 7R > 9R ~ 10R Specificity rank agrees with cell lysate Western Blots (Goodman et.al. Biol. Open 1:329)
Replicate mAb specific peptide lists exhibit high sequence overlap (Right Table).

De Novo Target and Epitope ID

Proteome Alignment of Significant Array Peptides Can Identify mAb Target(s)

A

Coverage of FGFR1 aa 820-840 by array peptides

Amino acids 820-840 of FGFR1

B

Anti-FGFR1 6R mAb de novo target/epitope prediction

C

Putative Protein Target Hit	Epitope Hit Position	Alignment Score
FGFR1	820	901
FREM1	610	882
ZC3H4	550	868
NOX4	240	868
PRDM4	220	866

The top 100 dose-responsive peptides were aligned to the human proteome using a modified BLAST strategy and scoring matrix designed to reflect the composition of the array. (A) Coverage of each position across the proteome was calculated by summing each peptide's contribution to each protein's BLAST score at each amino acid. Target and epitope prediction occurs by summing this coverage score over tiling 20-mers across the proteome, yielding an alignment score for each protein/epitope (B), with the known epitope as the top hit (C).

Important findings from the Merck KGaA mAb specificity study on HealthTell's platform are: 1) Different mAb clones raised against the same immunogen produce distinct binding specificity profiles in diverse sequence space, 2) Array binding profiles can map to the known immunogen and each clone has a unique profile of significant binding peptide enriched motifs and sequence conservation, 3) Array binding profiles can identify the antibody target from a proteome database search, 4) Blinded mAb replicates demonstrate that the HealthTell platform binding profiles are reproducible.

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