Faster Generation of Anti-Drug Antibodies Using SpyTag Technology

Dr. Sarah-Jane Kellmann, Scientist R&D, Bio-Rad Laboratories, Inc

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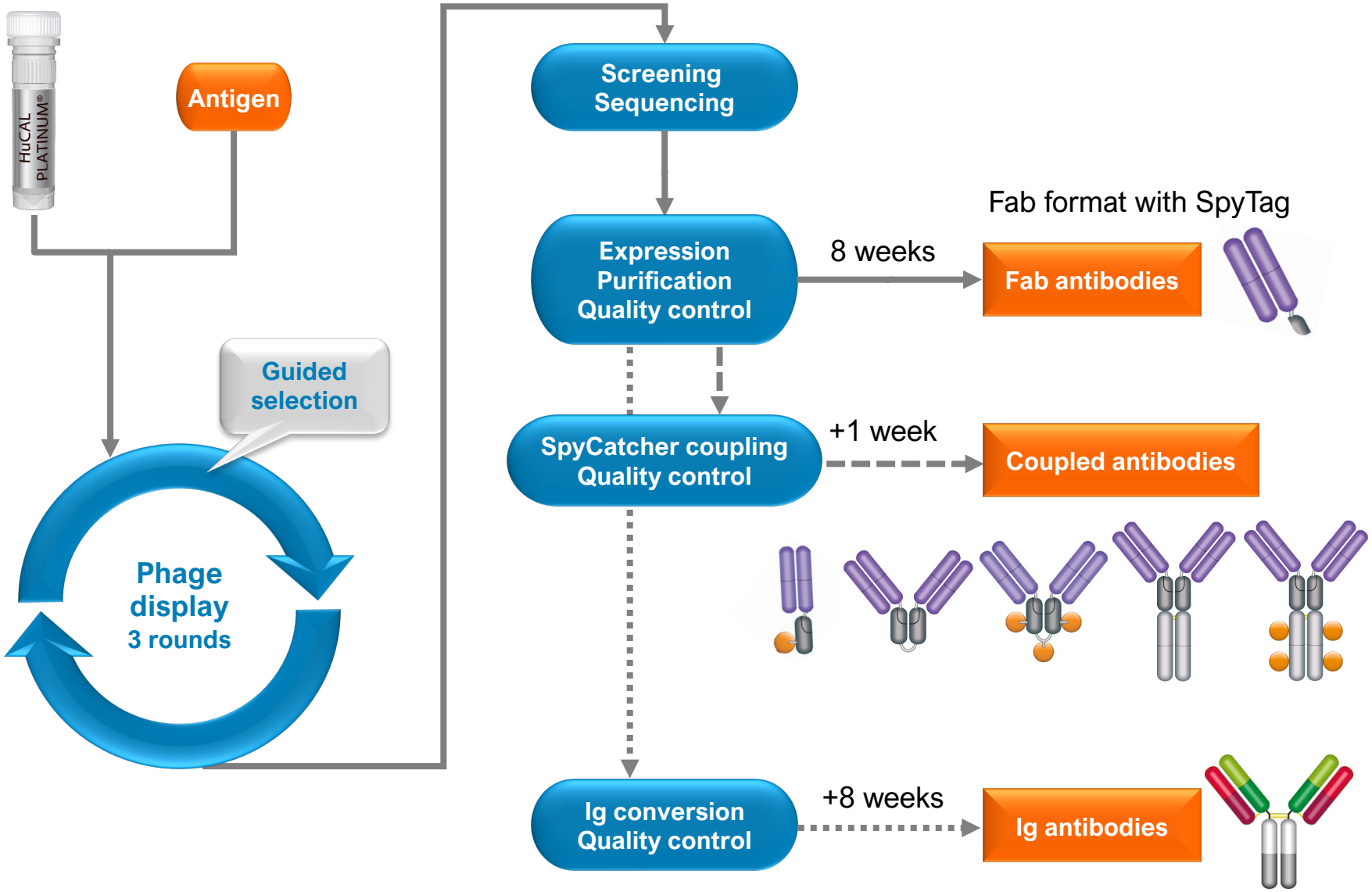
In Vitro Antibody Generation

- Generation of monoclonal antibodies for research and in vitro diagnostic use
- HuCAL PLATINUM® phage display technology
- Well-established automated procedures
- Fab antibodies ready in 8 weeks
- Over 17 years of experience
- Generation of almost 50,000 different antibodies



SpyTag
Modular Antibody Assembly Platform

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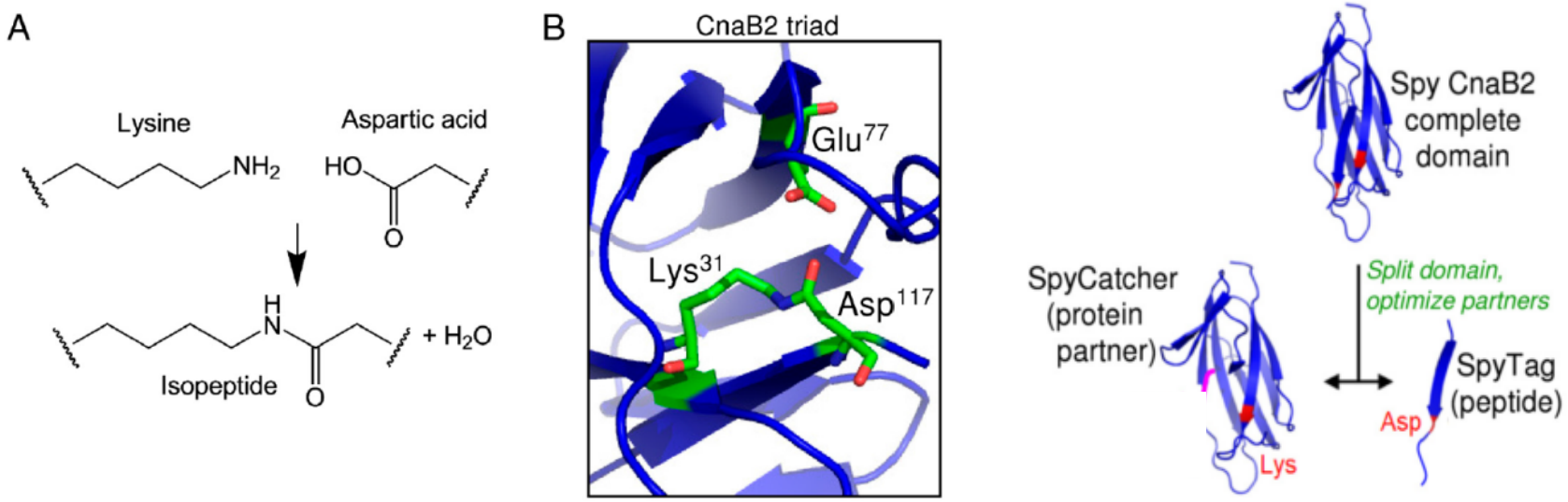
SpyTag Technology

- Based on *Streptococcus pyogenes* fibronectin-binding protein FbaB
- Contains spontaneous isopeptide bond between Lys and Asp



Zakeri B et al. (2012). Peptide tag forming a rapid covalent bond to a protein, through engineering a bacterial adhesin. PNAS 109, E:690-697.

SpyTag-SpyCatcher — Fast, Spontaneous Reaction

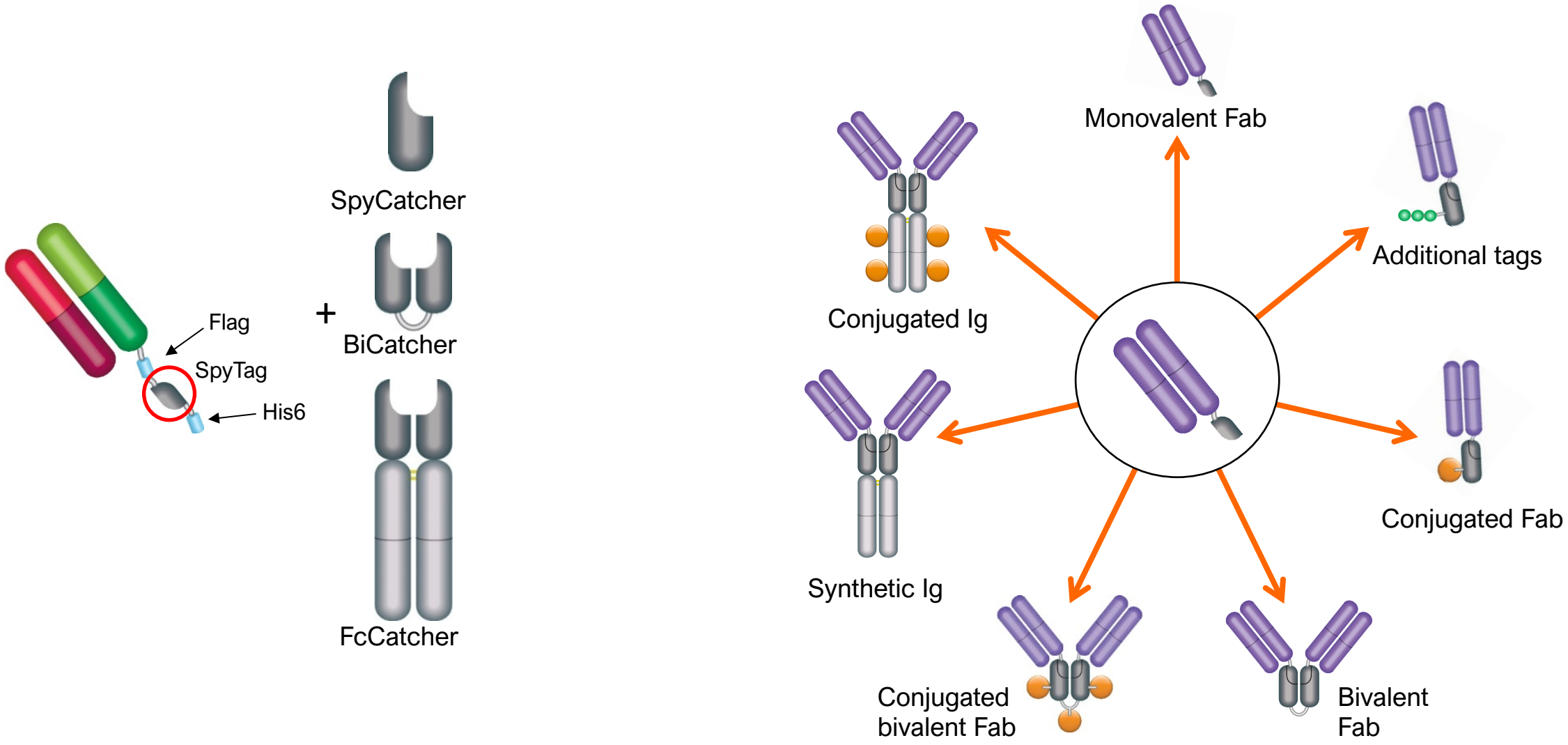
Ligated proteins

<u>SpyCatcher2</u>
- 113 aa
- 12.08 kDa
- No cysteine

<u>SpyTag2</u>
- 14 aa
- Reactive when at N- or C-terminus or internal

- Spontaneous (autocatalytic) reaction
- Covalent isopeptide bond formation, irreversible
- Fast, quantitative reaction
- pH 5–8, temperature +4–37°C
- Robust to buffer conditions, Ca^{2+}/Mg^{2+} not needed
- Robust to detergents
- Reaction occurs also inside cells (in vivo)

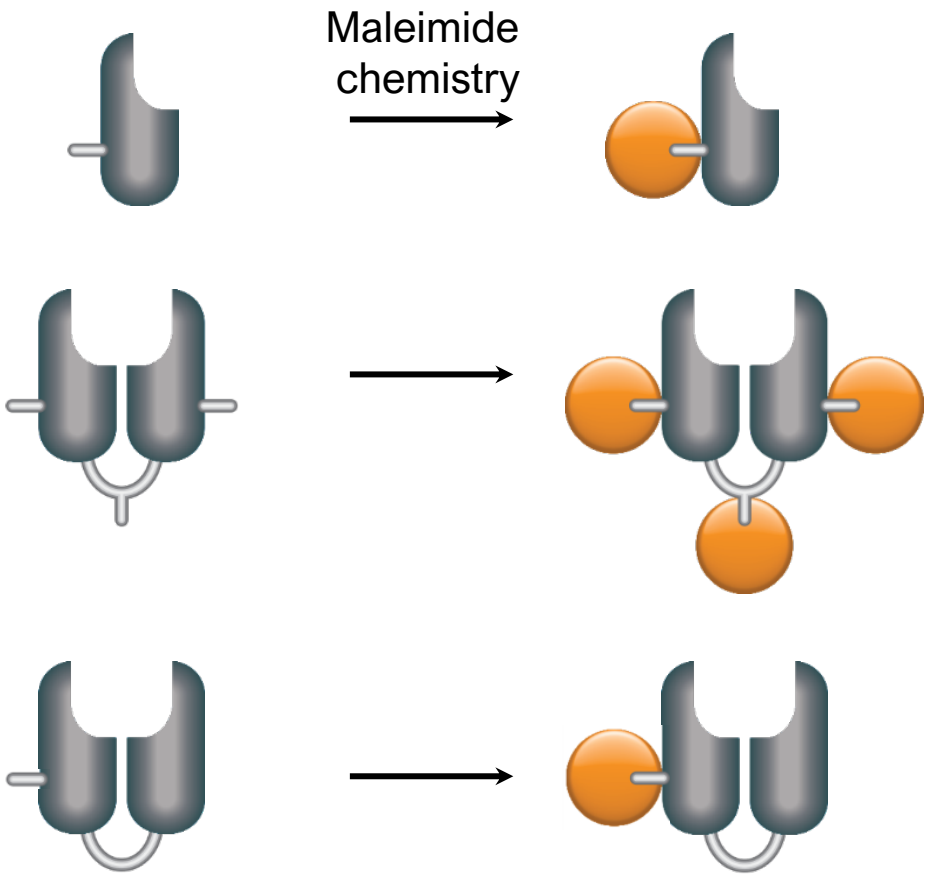
One Antibody, Multiple Formats in Less Than One Hour



Hentrich et al. (2021). Periplasmic expression of SpyTagged antibody fragments enables rapid modular antibody assembly. Cell Chemical Biology 28, 1–12.

Site-Specific Conjugation

- Cysteines introduced into SpyCatcher
- Site-specific labeling
- Controlled degree of labeling
- High batch-to-batch consistency
- No modification of antibody binding site



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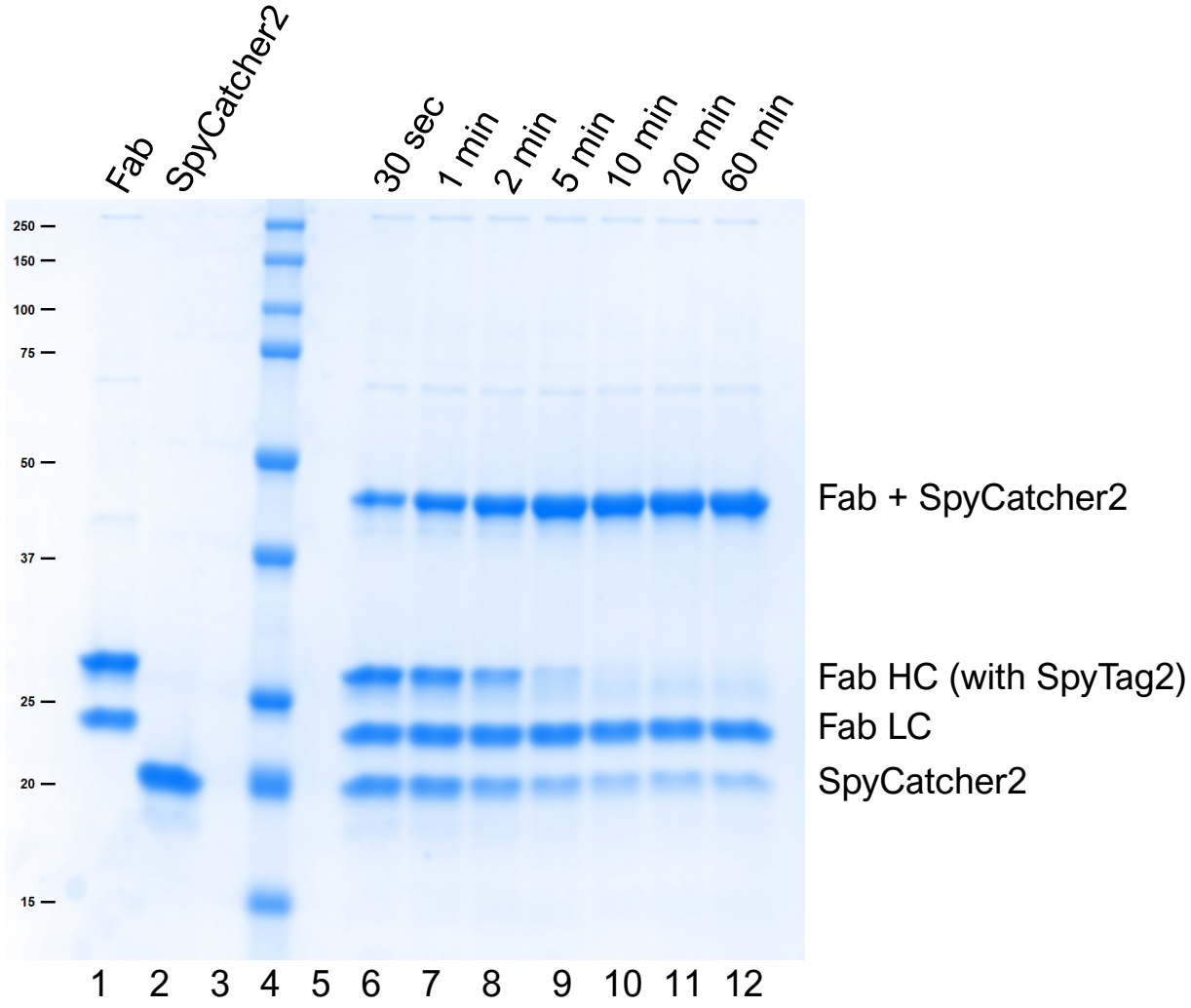
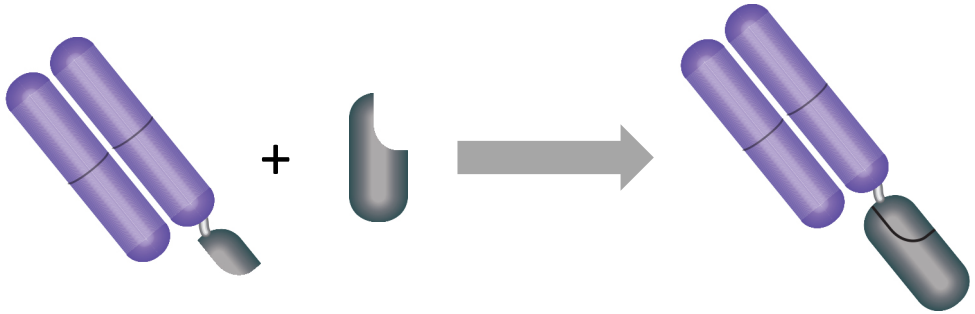
SpyTag Fab and SpyCatcher Coupling Reaction



Coupling at room temperature
Ratio Fab : Catcher = 2.5:1

AnyKD Criterion Gel
Nonreduced samples
3 µg protein loaded per lane



Generation of Anti-Idiotypic Antibodies

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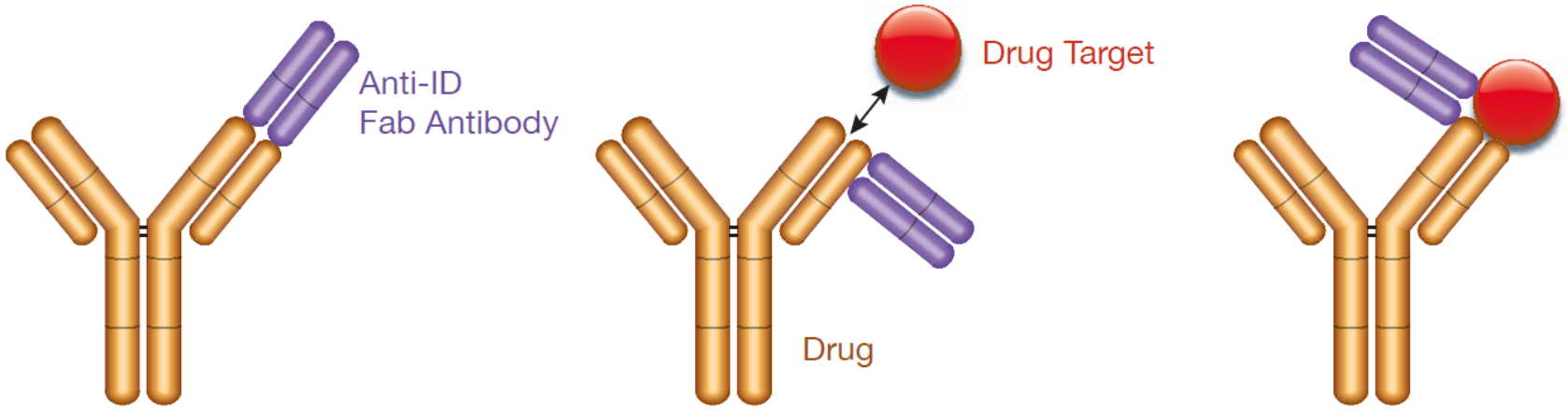
Anti-Idiotypic Antibodies



Type 1

- Anti-idiotypic antibody
- Paratope–specific
- Inhibitory
- Neutralizing
- Detects free drug

Type 2

- Anti-idiotypic antibody
- Not paratope–specific
- Not inhibitory
- Detects total drug (free, partially bound, fully bound)

Type 3

- Drug target complex–specific
- Not inhibitory
- Detects bound drug exclusively

A Proven Workflow for Anti-Idiotypic Antibody Generation

Selection strategies to generate binding types 1, 2, and 3

Additional characterization

- Affinity ranking and measurement
- Selection of best pairs

Extended quality control to meet critical reagent requirements

Expertise demonstrated with a portfolio of ready-made anti-biotherapeutic antibodies

Autoimmune disease

- Adalimumab (Humira)
- Certolizumab pegol (Cimzia)
- Etanercept (Enbrel)
- Golimumab (Simponi)
- Infliximab (Remicade)
- Rituximab (Rituxan)
- Tocilizumab (Actemra)
- Vedolizumab (Entyvio)
- Ustekinumab (Stelara)

Age-related macular degeneration

- Ranibizumab (Lucentis)

Allergic asthma

- Omalizumab (Xolair)

Respiratory disease

- Palivizumab (Synagis)

Cancer

- Alemtuzumab (Campath)
- Bevacizumab (Avastin)
- Cetuximab (Erbitux)
- Ipilimumab (Yervoy)
- Nivolumab (Opdivo)
- Panitumumab (Vectibix)
- Pembrolizumab (Keytruda)
- Trastuzumab (Herceptin)

Multiple sclerosis

- Natalizumab (Tysabri)

Osteoporosis

- Denosumab (Prolia)

PNH, aHUS (rare diseases)

- Eculizumab (Soliris)

bio-rad-antibodies.com/biotherapeutics

https://www.bio-rad-antibodies.com/biotherapeutic-antibodies-bioanalysis-drug-monitoring.html

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Generation of Anti-Drug Antibodies

- Antibodies specific for biotherapeutic drugs play an important role in preclinical and clinical development
- Used for pharmacokinetic (PK) studies and for the development of immunogenicity assays
- Advantages of integrating SpyTag technology
    - Rapid site-specific labeling
    - Conversion into synthetic IgG in one hour

- Example: Daratumumab (Darzalex)
    - → Fully human IgG1 kappa
    - → Generated with transgenic mouse technology
    - → Developed by Genmab, distributed by Janssen Biotech
    - → Treatment of multiple myeloma
    - → Binds CD38, causing cells to apoptose

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Anti-Daratumumab Antibodies Project — Primary Screening

Compute

Clear

Auto Fill

Plate:	Plate1	Plate2	Plate3	Plate4	Plate5	Plate6	Plate7	Plate8	Plate9	Plate10
Plate ID:	444.12A 10890-1	444.12A 10889-1+	444.12A 08060-1-\	Fab-F-Spy2-H	E44.12A 10890-1+	I44.12A 08060-1- I				
Threshold:	>= 5 x bg	>= 5 x bg	>= 2 x bg							
Role:	Reference	Positive	Negative							

Result Compute: P1(#), P2(+), P3(-)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	0	0	40353	1567	43679	0	0	0	0	0	0	0	0	23763	38994	7392	0	0	0	3517	6158	0	0	0
B	0	9483	0	0	0	0	0	0	0	38085	0	0	17018	0	0	0	0	0	0	0	0	0	0	0
C	0	0	41644	0	0	0	0	0	0	43656	0	0	0	0	0	0	2734	0	37905	0	0	1932	0	0
D	0	0	0	0	0	0	37913	39351	2367	0	0	40429	0	0	0	0	0	0	0	0	0	0	0	0
E	0	0	0	0	0	0	0	0	0	0	0	0	39879	1762	0	0	0	0	2647	0	0	0	0	0
F	0	0	0	39496	0	0	0	2122	0	0	0	0	0	0	5027	0	0	0	6055	0	37480	0	0	0
G	0	11895	0	0	0	0	2789	41189	30433	42004	0	0	0	0	40560	2246	0	0	0	45476	0	0	0	0
H	0	0	0	0	0	0	0	0	2676	0	0	0	0	0	0	0	41824	0	0	12557	20958	0	2221	0
I	0	0	0	0	0	0	0	4341	0	0	0	0	0	0	0	38690	0	0	0	0	0	0	30731	0
J	0	43315	0	0	0	0	0	0	0	33530	0	0	2931	0	0	12053	0	0	0	0	38554	25420	0	0
K	0	0	0	0	0	0	0	0	0	0	0	0	39125	0	0	0	0	0	0	0	0	5047	0	0
L	0	0	42256	0	0	0	23122	0	0	0	5519	0	14109	0	0	35633	0	0	0	15454	7037	25003	0	0
M	0	0	0	41185	37320	10185	0	0	0	0	32944	0	0	0	0	0	0	0	0	0	9238	6544	0	0
N	37499	1735	15567	0	0	0	0	0	0	0	0	0	2973	0	0	0	5577	0	0	0	0	0	13208	0
O	38425	31114	8049	0	0	0	0	11936	0	7446	0	0	0	0	0	0	0	0	0	0	26641	2123	0	0
P	0	0	0	0	0	37370	0	0	0	0	0	0	39657	1749	0	0	0	0	0	0	0	0	16874	0

Green: Signal 10-fold above background (73/368, 20%)

Yellow: Signal 5-fold above background

18 clones sequenced → 12 different antibodies

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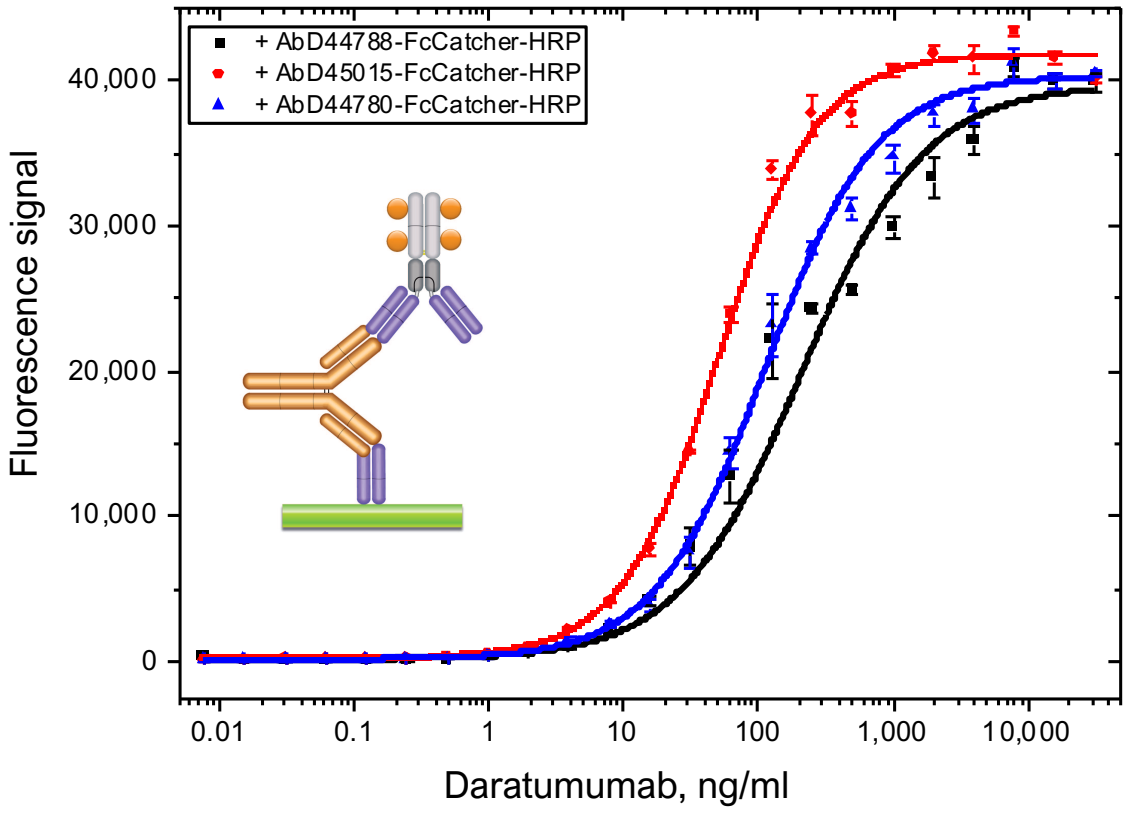
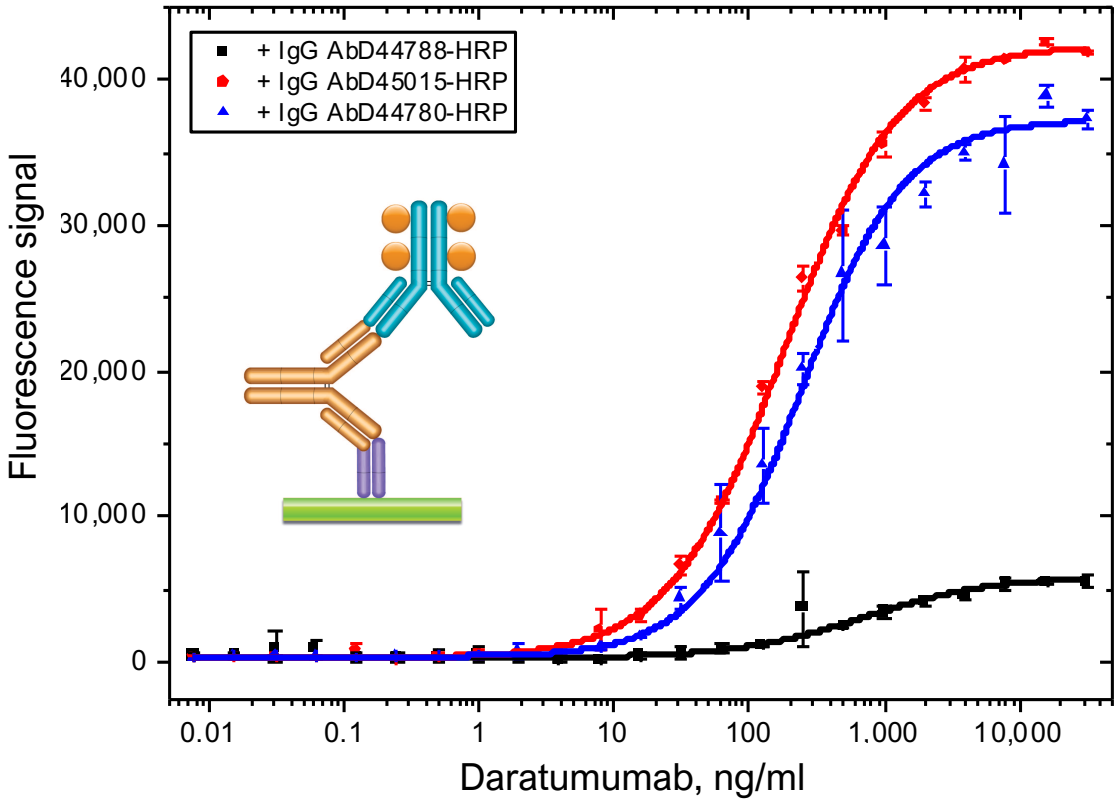
Comparison of Antibody Format Performance in PK Bridging ELISA

Fab-Spy-FcCatcher-HRP



IgG-HRP



- Similar performance of IgG and FcCatcher formats
- Higher sensitivity with FcCatcher due to higher labeling efficiency (site-specific!)

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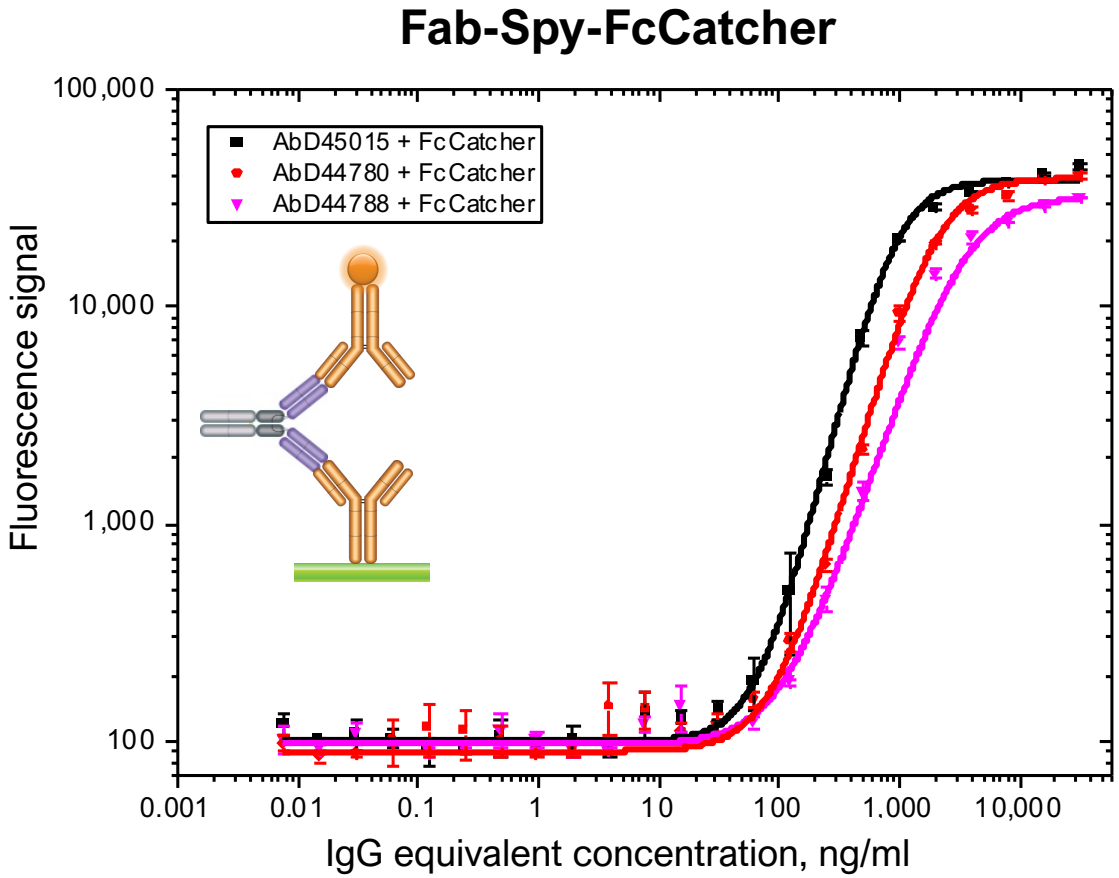
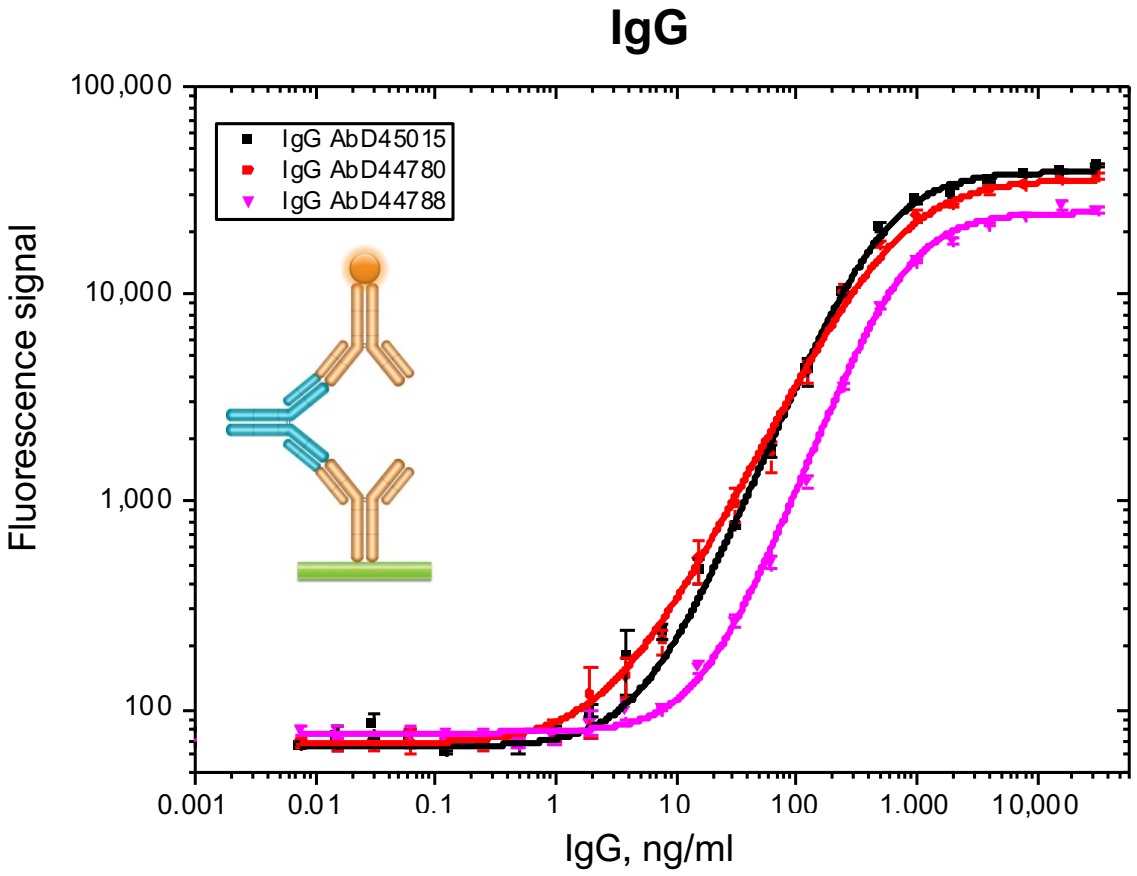
Comparison of Antibody Format Performance in ADA Bridging ELISA





- Performance of anti-daratumumab antibodies compares well across FcCatcher and IgG formats
- Small scale coupling reaction for many clones possible
- Rapid conversion of many clones and fast identification of most promising candidates

  → Faster, more economical, and more candidates tested than in the conventional workflow

Summary — Recombinant Antibodies with SpyTag Technology

- Fast and robust site-specific labeling of antibodies
- Access to bivalent Fabs or synthetic IgGs within one hour

  → Rapid conversion of many clones, and fast identification of most promising candidates
- Fast and easy switching of species or isotype
- Similar or better performance than corresponding controls in various assays (e.g., ADA, PK)
- Defined product
  - Controlled degree of labeling
  - High batch-to-batch consistency
  - No modification of antibody binding site
- One recombinant Fab gives access to a multitude of experimental setups

  → Choose the best matching Catcher from an “engineering toolbox”

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Visit bio-rad-antibodies.com/TrailBlazer for more information.