



Drug-Target-Complex Specific Antibodies for Pharmacokinetic Analysis of Biotherapeutics

BIO-RAD

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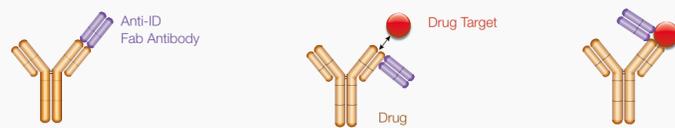
1 Introduction

Anti-idiotypic antibodies play an important role in preclinical and clinical development of therapeutic antibodies, where they are used for pharmacokinetic studies and for the development of immunogenicity assays.

By using the fully synthetic Human Combinatorial Antibody Library, HuCAL PLATINUM®, in combination with in vitro guided selection against various marketed drugs, we have generated antibodies that recognize the drug only when bound to its target. We have named such specificities Type 3, to distinguish them from the anti-idiotypic antibodies that either specifically detect free antibody drug (Type 1) or total drug (Type 2), Figure 1.

We describe the generation and characterization of such reagents for the development of drug monitoring assays. We also show how these Type 3 antibodies can be used to develop very specific and sensitive assays that avoid the bridging ELISA format.

Types of Anti-Biotherapeutic Antibodies



Type 1

- Anti-idiotypic (Anti-ID) 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

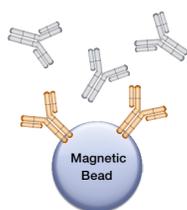
- Drug-target complex specific
- Not inhibitory
- Detects bound drug exclusively

Fig. 1. Binding modes and properties of anti-biotherapeutic antibodies.

2 Generation of Type 1, 2 and 3 Antibodies by Phage Display

Type 1

Selection on drug (yellow), blocking with isotype matched control antibodies (gray), plus human serum.



Type 2 and Type 3

Selection on drug-target complex, blocking with isotype matched control antibodies and with target antigen (red), plus human serum.

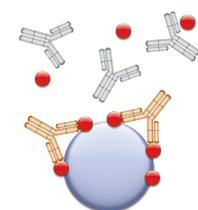
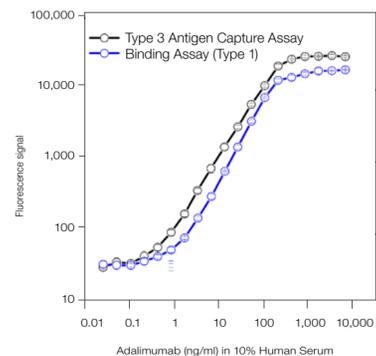


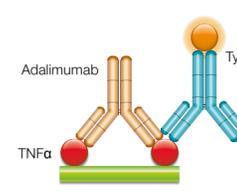
Fig. 2. Guided selection strategies for generation of Type 1, 2 and 3 anti-biotherapeutic antibodies by phage display. For Type 1 antibodies, selection on the drug in the presence of isotype subclass matched antibodies avoids enrichment of specificities that bind to the constant regions of the antibody drug. For Type 2 and Type 3 antibodies, selection is on the drug-target complex, blocking is with isotype matched control antibodies and target antigen.

3 Pharmacokinetic (PK) Antigen Capture Assay

The PK antigen capture assay using a Type 3 antibody shows higher sensitivity than a PK bridging assay, without the need for a high affinity reagent.



Antigen Capture Assay (Type 3)



Bridging Assay (Type 1)

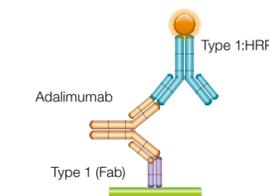


Fig. 3. Detection of adalimumab using a Type 3 antibody in antigen capture assay (black), and Type 1 capture and detection antibodies in a bridging assay (blue).

Antigen Capture Assay: Human TNF α was coated at 5 μ g/ml on a microtiter plate overnight. After washing and blocking, adalimumab spiked into 10% human serum was added. HRP conjugated Anti-Adalimumab/TNF α Antibody catalog #HCA207 (Type 3, affinity 67 nM) was added at 2 μ g/ml in HISPEC Assay Diluent, followed by QuantaBlu Fluorogenic Peroxidase Substrate.

Adalimumab Bridging Assay: Anti-Adalimumab Antibody, #HCA202 (Type 1, affinity 0.16 nM) was coated at 1 μ g/ml on a microtiter plate overnight. After washing and blocking, adalimumab spiked into 10% human serum was added, followed by HRP conjugated Anti-Adalimumab Antibody, #HCA204 (Type 1, affinity 0.06 nM), at 2 μ g/ml in HISPEC Assay Diluent and QuantaBlu Fluorogenic Peroxidase Substrate.

Table 1. Specifications of Type 3 Anti-Biotherapeutic Antibodies

Specificity	Clone	Formats (a)	Affinity on Complex (K _d nM) (b)	Catalog #
Adalimumab/TNF α	AbD18754	Fab-FH, IgG1	67 nM	HCA206, HCA207
	AbD20350	Fab-FH	3 nM	HCA231
	AbD20349	IgG1	7 nM	HCA232
Golimumab/TNF α	AbD20893	IgG1	53 nM	HCA245
	AbD25705	Fab-FH	6 nM	HCA274
Omalizumab/hIgE	AbD20760	Fab-FH	0.6 nM	HCA238
		IgG1		HCA237
Trastuzumab/HER2	AbD25279	Fab-A-FH	31 nM	HCA263
Ranibizumab/VEGF	AbD29928	Fab-FH	1 nM	N.A.

(a) Fab-FH, monovalent Fab fragment with His-tag and DYKDDDDK-tag; IgG1, full-length human IgG1; Fab-A-FH, bivalent Fab fragment fused to bacterial alkaline phosphatase (BAP), with His-tag and DYKDDDDK-tag. (b) Affinities were measured with real-time kinetics on the drug-target complex using monovalent Fab fragments.

4 Type 3 Antibody Specificity

Type 3 complex-specific antibodies are highly specific for the complex and do not bind free drug or free drug target.

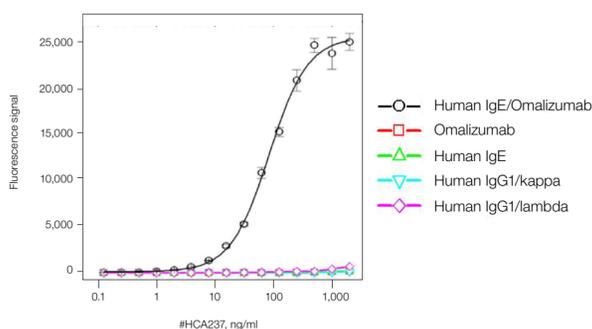


Fig. 4. Demonstration of Type 3 antibody specificity. A microtiter plate was coated overnight with human IgE, omalizumab, human IgG1/kappa or human IgG1/lambda at a concentration of 5 μ g/ml. After washing and blocking with PBST + 5% BSA, the omalizumab/hIgE complex was formed by adding 2 μ g/ml omalizumab to the wells coated with IgE. Detection with HRP conjugated Anti-Omalizumab/hIgE Antibody, #HCA237 in PBST, followed by QuantaBlu Fluorogenic Peroxidase Substrate.

5 Assay Design Flexibility

The PK antigen capture assay with a Type 3 antibody allows various secondary reagents for detection.

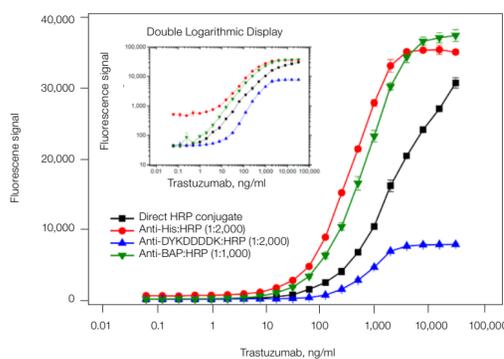


Fig. 5. Detection of trastuzumab in 10% human serum using a Type 3 antibody (monovalent Fab). Human HER2 was coated at 5 μ g/ml on a microtiter plate overnight. After washing and blocking, trastuzumab spiked into 10% human serum was added. Complex-specific Anti-Trastuzumab/HER2 Antibody, #HCA263, was added at 2 μ g/ml in HISPEC Assay Diluent, either directly HRP conjugated (black), or followed by an HRP conjugated secondary antibody. All three secondary antibodies tested can be used for detection, as well as the directly HRP conjugated anti-drug antibody. Both Anti-Penta-Histidine-tag Antibody #MCA595P and Anti-BAP Antibody #HCA275P result in higher assay sensitivity. The Anti-BAP Antibody produced less background signal (see inset).

6 PK Assay Insensitivity to Serum Components

The PK antigen capture assay with a Type 3 antibody is insensitive to serum components.

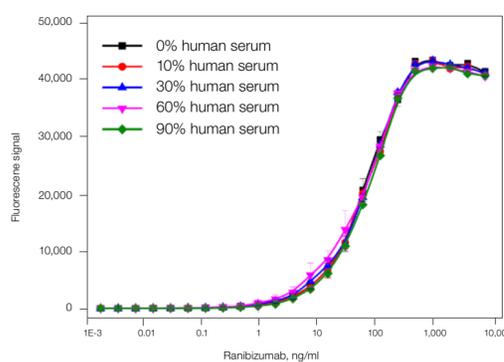


Fig. 6. Detection of ranibizumab (Fab format antibody drug) in different serum concentrations using a Type 3 antibody. VEGF-A was coated at 5 μ g/ml on a microtiter plate overnight. After washing and blocking, ranibizumab spiked into different concentrations of normal human serum was added in increasing amounts and incubated for 1 hr at RT. Detection with HRP conjugated Anti-Ranibizumab Antibody, clone AbD29928 (Type 3, affinity 1 nM) in HISPEC Assay Diluent.

7 Sensitive Detection of Drug-Target Complexes by Type 3 Antibodies

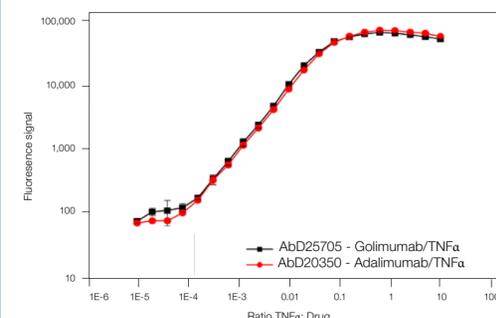


Fig. 7. Detection of drug/TNF α complexes by Type 3 antibodies in monovalent Fab format. Type 3 Anti-Golimumab/TNF α Antibody, #HCA274, and Type 3 Anti-Adalimumab/TNF α Antibody, #HCA231 were coated on a microtiter plate at 1 μ g/ml. Golimumab and adalimumab at a fixed concentration (1 μ g/ml = 7 nM) were incubated for 1 hr with an increasing amount of TNF α and added to the plate. Detection with Anti-Human IgG (Fc) CH2 Domain:HRP #MCA647P. Both Type 3 antibodies detect the drug-target complexes down to a ratio of target:drug of 1:10,000.

8 Detection of Drug-Target Complexes with Type 1, 2 or 3 Antibodies Reveal Very Distinct Curves

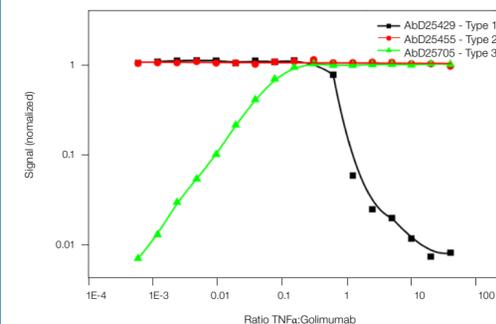


Fig. 8. Detection of golimumab/TNF α complex by Type 1, 2 and 3 Fab format antibodies. The Anti-Golimumab/TNF α Antibodies, Type 1 #HCA286, Type 2 #HCA289 and Type 3 #HCA274, were coated on a microtiter plate. Golimumab at fixed concentration (300 ng/ml = 2 nM) was incubated for 1 hr with an increasing amount of TNF α and added to the plate. Detection with Anti-Human IgG (Fc) CH2 Domain:HRP #MCA647P that detects golimumab but not the Fab antibodies. Type 2 detects drug regardless of the amount of TNF α present; Type 1 shows a reduction of signal at a ratio of about 0.4 (TNF α :golimumab); Type 3 detects the drug-target complex at low ratios and reaches saturation when the ratio of TNF α to golimumab reaches about 0.4.

9 Results and Conclusions

We isolated antibodies from the HuCAL PLATINUM recombinant synthetic antibody library that are highly specific to drug-target complexes and named them Type 3 anti-biotherapeutic antibodies (Figures 1 and 4). Type 3 antibodies can be used to develop PK assays with high sensitivities that avoid the bridging assay format (Figure 3). The Type 3 antigen capture assay (Figure 3) results in a high sensitivity with a lower affinity reagent (1,200-fold lower as compared to the Type 1 detection reagent). A range of secondary reagents is available for detection of Type 3 antibodies that include a tag (Figure 5). Figure 6 shows detection of the drug ranibizumab with a Type 3 reagent. Ranibizumab is a Fab fragment and therefore cannot be detected by a bridging assay. We show that the assay performance is not influenced by matrix components in up to 90% human serum. Type 3 antibodies bind the drug-target complexes only. Such complexes can be detected at very low concentrations. For two Type 3 reagents we show that complexes are detected even when the free drug is present in 10,000 fold higher concentrations (Figure 7). We compare the binding curves of Type 1, 2 and 3 reagents for golimumab detection, when the ratio of target to drug is increased (Figure 8). The curves reveal the different specificities; Type 1 binds free drug until all drug is complexed with the target, Type 2 always binds to the drug regardless of the amount of drug target present; Type 3 detects drug-target complexes until saturation is reached, i.e. all drug is bound to target.

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