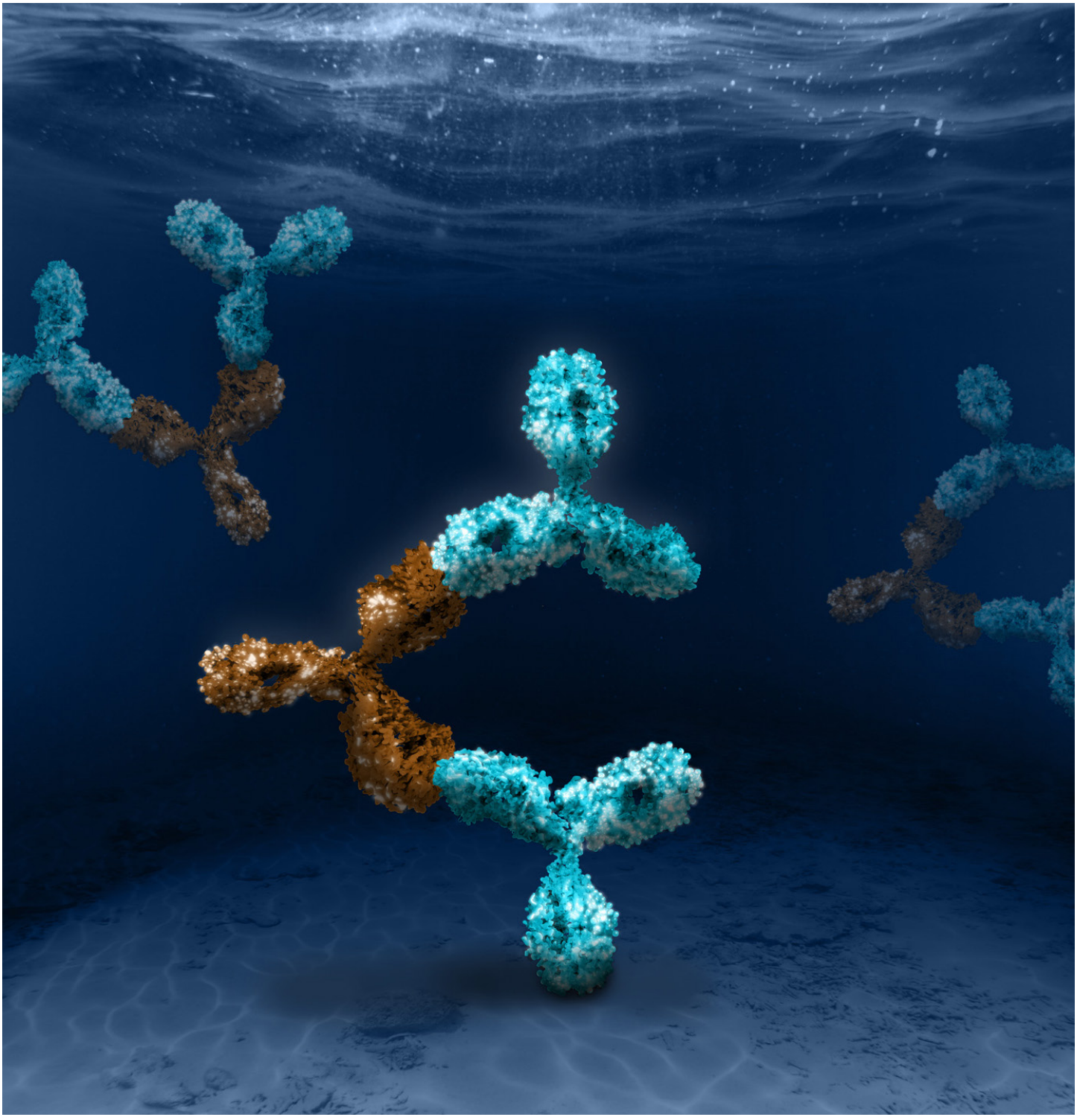
Biotherapeutics

Antibodies to Biotherapeutics

Supporting Bioanalytical Assay Development



Visit **bio-rad-antibodies.com/biotherapeutics** for more information.

BIO-RAD

Ready Made Antibodies to Biotherapeutics

Bio-Rad has developed highly specific, high affinity anti-biotherapeutic antibodies to support preclinical research, clinical trials, and patient monitoring for innovator and biosimilar products.

Select antibodies to your target from our industry-leading range of antibodies against marketed biotherapeutic monoclonal antibody drugs, or commission a custom made antibody against your proprietary biotherapeutic lead.

Special features of our ready made antibodies include:

- Application data and protocols to support pharmacokinetic (PK), anti-drug antibody (ADA), and inhibition assays
- High specificity and sensitivity for optimal assay development
- Specialized antibodies binding to a complex of the drug and target
- Recommended ELISA detection antibodies available directly conjugated to HRP
- Fully human immunoglobulins as controls and calibrators for ADA assays
- Unlimited and consistent supply throughout preclinical and clinical studies

Table 1. Anti-biotherapeutic antibody range.

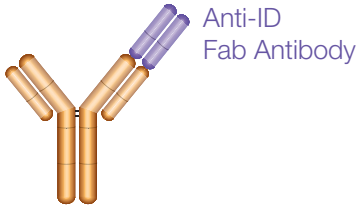
Antibody Target INN/Trade Name	Number of Products Available		Binding Modes Available			Affinities Available[1] (K_D, nM)	Supporting Data		
	Fab	Ig	Inhibitory	Noninhibitory	Complex Binder		PK/PD	ADA	Inhibition
Abatacept/Orencia		2 IgG1	•			6–1	•	•	•
Adalimumab/Humira	3	5 IgG1 1 IgE	•		•	67.0–0.06	•	•	•
Alemtuzumab/Lemtrada	2	2 IgG1	•			2.4–0.2	•	•	•
Bevacizumab/Avastin	1	2 IgG1	•			2.0–0.4	•	•	
Certolizumab pegol/Cimzia		3 IgG1	•			3–1		•	•
Cetuximab/Erbitux	1	4 IgG1	•	•		13.5–0.5	•	•	•
Denosumab/Prolia	1	3 IgG1	•			7.1–0.8	•	•	•
Eculizumab/Soliris	1	3 IgG1	•			6–0.6	•	•	•
Etanercept/Enbrel	1	1 IgG1 1 IgG4	•	•[2]		14.8–2.4	•	•	•
Golimumab/Simponi	5	5 IgG1	•	•	•	53–0.1	•	•	•
Infliximab/Remicade	3	4 IgG1	•	•		3.9–0.12	•	•	•
Ipilimumab/Yervoy	2	3 IgG1	•		•	252–0.3	•	•	•
Natalizumab/Tysabri	1	2 IgG1	•			12.2–2.1	•	•	•
Nivolumab/Opdivo	1	4 IgG1	•			23–0.5	•	•	•
Omalizumab/Xolair	2	2 IgG1	•		•	1.1–0.58	•	•	•
Palivizumab/Synagis	1	1 IgG1	•			1.5	•	•	
Panitumumab/Vectibix	1	2 IgG1	•			0.6–0.3	•	•	•
Pembrolizumab/Keytruda	1	2 IgG1	•			5–0.6	•	•	•
Ranibizumab/Lucentis	3	2 IgG1	•	•	•	18–0.4	•	•	
Rituximab/Rituxan	3	1 IgG1	•			10.0–0.13	•	•	
Tocilizumab/Actemra	1	5 IgG1	•	•		31.0–0.1	•	•	•
Trastuzumab/Herceptin	5	2 IgG1 1 IgG4	•		•	2.5–0.02	•	•	•
Ustekinumab/Stelara	2	2 IgG1	•			2.8–0.2	•	•	•
Vedolizumab/Entyvio	1	3 IgG1	•			4–0.1	•	•	•

[1] Given as a range; Lowest affinity antibody available — highest affinity antibody available. NB. Affinities are measured in the monovalent Fab format.
[2] One noninhibitory antibody recognizes the TNFR2 domain; a second noninhibitory antibody is specific for the TNFR2-Fc fusion region of etanercept.

Antibodies Designed for Assay Optimization

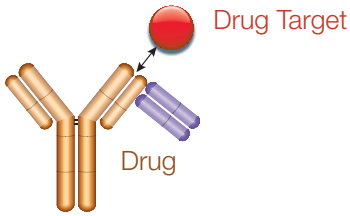
Different Binding Modes of Anti-Biotherapeutic Antibodies

Expertly designed guided selection strategies allow the development of three types of anti-biotherapeutic antibody.



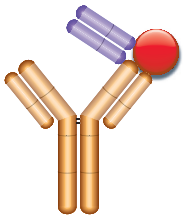
Type 1

- 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)

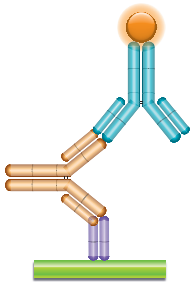


Type 3

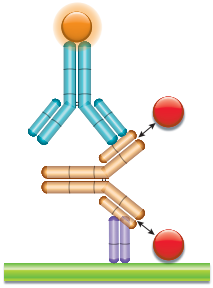
- Drug target complex-specific
- Not inhibitory
- Detects bound drug exclusively

Examples of Assay Formats with the Different Types of Antibodies

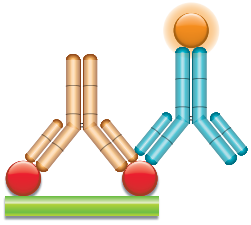
The use of highly specific, high affinity Type 1, Type 2, and Type 3 antibodies gives flexibility to the assay developer and enables sensitive and robust assays to be built, leading to improved pharmacokinetic studies.



Type 1 anti-idiotypic antibody bridging assay: a highly specific anti-ID antibody (purple, Fab) is immobilized at low density and captures the drug (gold) in the serum sample; the bound drug is quantified with a second labeled anti-ID antibody (blue, IgG) that binds to the free idiotope of the drug.



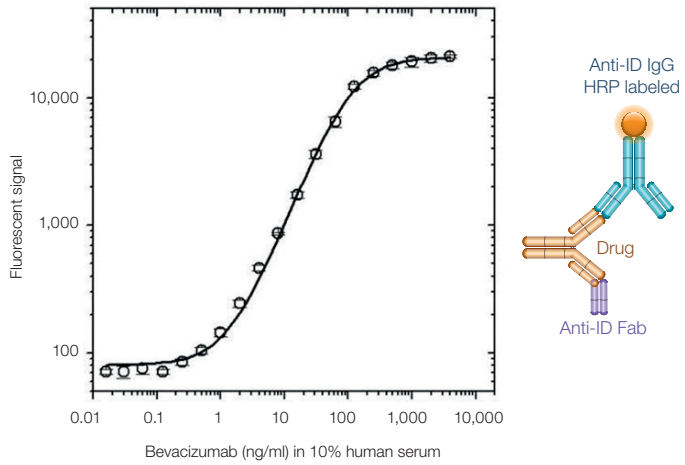
Type 2 anti-idiotypic antibody bridging assay: a highly specific Type 2 anti-ID antibody is immobilized at low density and captures both free drug and drug bound to its target (red circle) in the serum sample; the drug is quantified with a second labeled Type 2 anti-ID antibody that binds to the free idiotope of the drug; this assay measures total drug (free, partially bound, and fully bound).



Type 3 complex-specific antibody assay: the Type 3 antibody (blue, IgG) binds neither the antibody drug nor the target when on their own and can be used to detect bound therapeutic antibodies directly; with a Type 3 antibody the bridging format for PK studies is not necessary, reducing assay development time and increasing sensitivity.

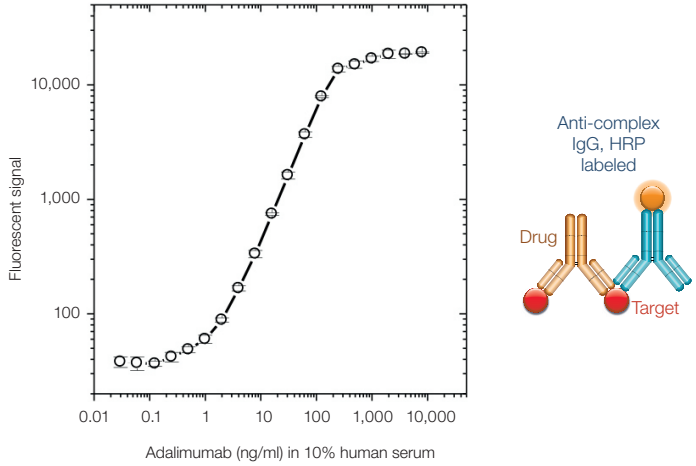
Characterized Assays Supported with Assay Data

PK Assay — Bridging ELISA Format Using Type 1 Antibodies



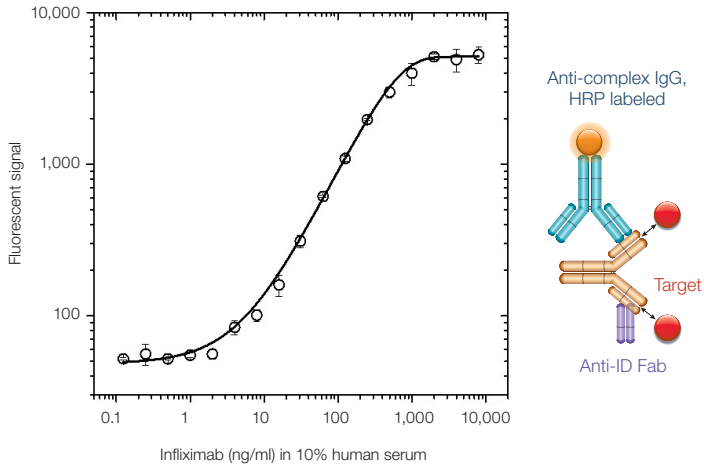
Anti-Bevacizumab Antibody (cat. #HCA182) was coated at 1.0 µg/ml on a microtiter plate overnight. After washing and blocking with 5% BSA in PBST, bevacizumab was added, spiked in the given concentrations into 10% human serum. Detection was performed by HRP conjugated Anti-Bevacizumab Antibody (#HCA184) (4.0 µg/ml in HISPEC Assay Diluent), plus QuantaBlu Fluorogenic Peroxidase Substrate.

PK Assay — Antigen Capture Format Using Type 3 Antibody



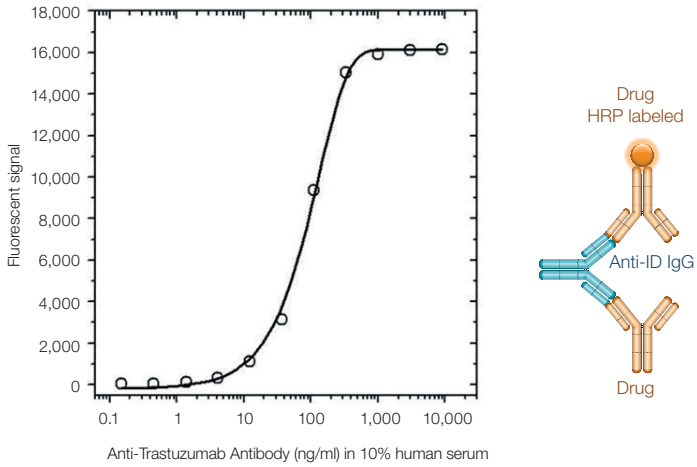
TNF-α was coated at 5.0 µg/ml on a microtiter plate overnight. After washing and blocking with 5% BSA in PBST, adalimumab (spiked in 10% human serum) was added in the concentrations indicated. Detection was performed using HRP conjugated drug target complex-specific Anti-Adalimumab Antibody #HCA207* (IgG1 format), at a concentration of 2.0 µg/ml in HISPEC Assay Diluent, plus QuantaBlu Fluorogenic Peroxidase Substrate.

PK Assay — Bridging ELISA Format Using Type 2 Antibodies



Anti-Infliximab Antibody, Type 2 (#HCA214) was used as the coating antibody (1.0 µg/ml) followed by infliximab spiked into 10% human serum as the antigen. Detection was performed by adding HRP conjugated Anti-Infliximab Antibody, Type 2 (#HCA216P, 2.0 µg/ml).

ADA Assay — Bridging Format Using Type 1 Human IgG Antibody



Trastuzumab was coated at 0.5 µg/ml on a microtiter plate overnight. Washing and blocking was performed with 5% BSA in PBST. Anti-Trastuzumab Antibody (#HCA177) (in PBST plus 10% human serum), was added in the given concentrations. Detection was performed by adding HRP conjugated trastuzumab*, (2.0 µg/ml in HISPEC Assay Diluent, #BUF049), plus QuantaBlu Fluorogenic Peroxidase Substrate.

*Antibodies were conjugated to HRP using a LYNX Rapid Conjugation Kit (cat #LNK001P - LNK006P).

Custom Service for Anti-Biotherapeutic Antibodies

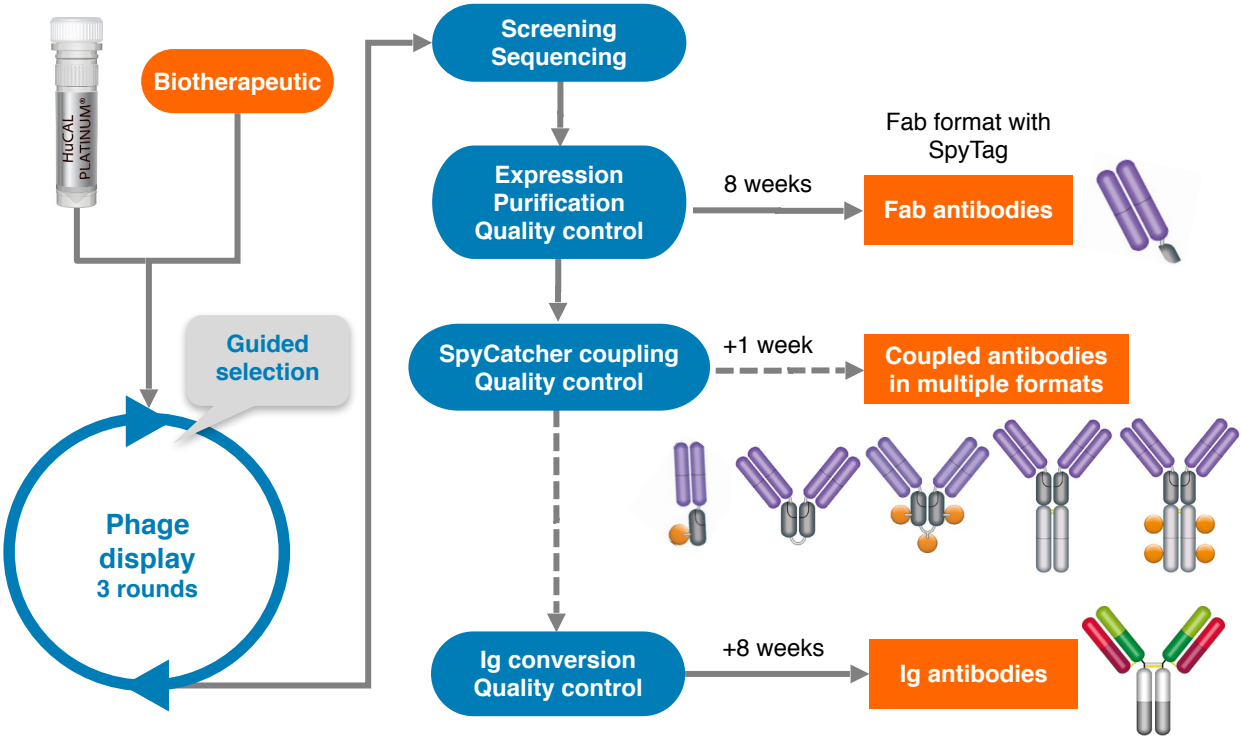
Bio-Rad provides a custom anti-biotherapeutic antibody generation service to support the preclinical and clinical development of biologics. We specialize in antibody generation projects to select high affinity, fully human anti-drug antibodies, ideal for use in PK and ADA assays. The service combines in vitro antibody phage display with SpyTag technology. Our recombinant antibodies have a SpyTag incorporated, which enables site-directed conjugation of antibodies to labels such as HRP and biotin, and rapid formation of bivalent and Ig-like constructs with a choice of isotypes.

This innovative combination of technologies brings you:

- Rapid generation of highly specific Fab antibodies in less than 3 months
- Isolation of antibodies against virtually any type of biologic, for instance antibody, fusion protein, CAR-T cell, ADC toxin, and DNA
- Guided selection in the presence of:
  - Isotype matched antibody, to ensure paratope specificity
  - Human serum, for avoidance of matrix effects
- Monovalent, bivalent, and conjugated formats for testing at the outset
- Conversion to fully human IgG, IgE, IgA, or IgM for use as a surrogate positive control
- Site-directed conjugation with consistent degree of labeling
- Additional characterization to select the best antibodies for your application
  - Affinity ranking and affinity measurement
  - Selection of best pairs for ELISA
  - Screening and quality control testing by flow cytometry
- Sequence identified antibodies with long-term secure supply guaranteed

We can design projects to select inhibitory, noninhibitory, and drug target complex-specific binders to your unique biologic.

- Save time — receive antibodies within 3 months
- Design better assays — test and optimize with different formats
- Improve experimental reproducibility — consistently label antibodies
- Generate robust data — have confidence in your results



Additional Products Supporting Drug Development and Bioanalysis

Anti-Human IgG Fc Specific Antibodies

Bio-Rad offers two antibody clones specific to human IgG Fc that can be used to detect human or humanized therapeutic antibodies in nonhuman primate samples.

Table 2. Anti-human IgG Fc specific antibody range.

Specificity	Clone	Catalog #	Format	Species Cross-Reactivity	Terms of Use
Human IgG1 Fc	AbD27686	HCA285 HCA285P	Fab-FH[1] Fab-FH[1] HRP labeled	Cynomolgus macaque — low cross-reactivity[2] Rhesus macaque — low cross-reactivity Baboon — not tested Marmoset — not tested Mouse IgG2a — no	Permitted for use for the detection of therapeutic antibodies from samples obtained from experimental animals
Human IgG CH2 domain	R10Z8E9	MCA5748G	Mouse IgG1	Cynomolgus macaque — no Rhesus macaque — no Baboon — no Marmoset — no Mouse — no Rat — no	Prior consent required for the detection of therapeutic antibodies from samples obtained from experimental animals[3]

[1] F=DYKDDDDK-tag H=His-6-tag.
[2] 89-fold lower affinity to cynomolgus macaque IgG than to human IgG1.
[3] Consent required by the owner of EP 1 853 921 (F. Hoffmann-La Roche AG, Basel, Switzerland) and /or US 7,955,806 (F. Hoffmann-La Roche, INC., Nutley, NJ).

Recombinant Human Immunoglobulin Isotype Controls

Isotype controls are used as negative controls to differentiate the nonspecific background signals initiated by the antibody Fc backbone or other cellular protein interactions, from the target-specific antibody signal.

- IgA, IgE, IgG1, IgG2, IgG3, IgG4, IgM
- Sequence of heavy and light chain known, defined allotypes
- Human cell line expressed for human glycosylation pattern
- IgG4 as wild-type and point mutant (S228P) to prevent the formation of IgG4 half molecules
- All antibodies bind specifically to GFP and are therefore negative on non-GFP containing matrices
- Source: HuCAL® phage display library; expression in human cell line

Table 3. Recombinant human immunoglobulin isotype control range.

Catalog #	Description
HCA189	Recombinant Human IgA1 Kappa
HCA172	Recombinant Human IgA1 Lambda
HCA190	Recombinant Human IgE Kappa
HCA171	Recombinant Human IgE Lambda
HCA192	Recombinant Human IgG1 Kappa Allotype G1m3
HCA319	Recombinant Human IgG1 Kappa Allotype G1m17,1
HCA049	Recombinant Human IgG1 Lambda Allotype G1m3
HCA318	Recombinant Human IgG1 Lambda Allotype G1m17,1
HCA193	Recombinant Human IgG2 Kappa
HCA108	Recombinant Human IgG2 Lambda
HCA337	Recombinant Human IgG2/4 Kappa
HCA194	Recombinant Human IgG3 Kappa
HCA178	Recombinant Human IgG3 Lambda
HCA195	Recombinant Human IgG4 Kappa
HCA050	Recombinant Human IgG4 Lambda
HCA247	Recombinant Human IgG4 (proline mutation) Kappa
HCA246	Recombinant Human IgG4 (proline mutation) Lambda
HCA191	Recombinant Human IgM Kappa
HCA170S	Recombinant Human IgM Lambda

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