

# Recombinant Engineered Antibodies

Superior Immunology Research Tools

ANTIBODY SEQUENCING | ANTIBODY ENGINEERING

ANTIBODY EXPRESSION | REAGENTS CATALOG

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science

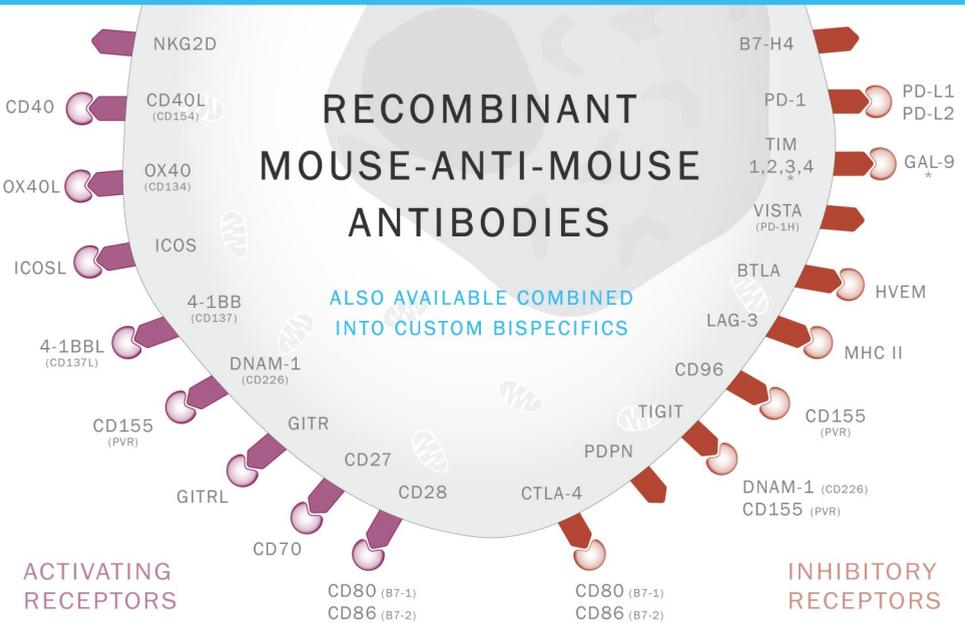
Your distributor in Switzerland

LubioScience GmbH  
Baumackerstrasse 24  
8050 Zürich  
Phone 041 417 02 80  
Fax 041 417 02 89

info@lubio.ch  
www.lubio.ch

**absolute**  
**antibody**

Your favorite research tools, improved with recombinant antibody technology.



- Established research tools, now updated through antibody engineering for improved performance *in vivo*
- Reduce immunogenicity by matching antibody species to your host organism
- Tailor effector function by choosing antibody isotype and subtype
- Combine two of these antibodies into a custom bispecific reagent

### See Also: Fc Fusion Proteins

Fc-Fusion proteins (also known as Fc chimeric fusion proteins, Fc-Ig, Ig-based Chimeric Fusion proteins and Fc-tag proteins) are composed of the Fc domain of IgG genetically linked to a peptide or protein of interest. Our Fc-Fusion proteins are matched to our collection of immunotherapy antibodies, with all products available in a range of species specific and engineered Fc domains.

## Recombinant Mouse-Anti-Mouse Antibodies

Catalog ID	Mouse Target	Antibody Clone	Original Format
Ab01052	4-1BB (CD137)	LOB12.3	Rat IgG1
Ab01054	4-1BBL (CD137L)	AT113-2	Rat IgG1
Ab00881	4-1BBL (CD137L)	TKS-1	Rat IgG2a
Ab01027	BTLA	HMBT-6B2	Hamster IgG
Ab00670	CD27	LG.3A10	Hamster IgG
Ab00895	CD27	RM27-3E5	Rat IgG2a
Ab00286	CD28	E18	Mouse IgG2b
Ab00285	CD28	D665	Mouse IgG1
Ab01109	CD40	Mar-23	Rat IgG2a
Ab01087	CD40L (CD154)	MR1	Hamster IgG
Ab00816	CD70	TAN 1-7	Rat IgG2a
Ab01056	CD70	TAN1-6	Rat IgG2a
Ab01028	CD80 (B7-1)	RM80	Rat IgG2a
Ab01029	CD86 (B7-2)	PO.3	Rat IgG2b
Ab00894	CTLA-4	9H10	Hamster IgG2
Ab01018	CTLA-4	9D9	Mouse IgG2b
Ab01088	GAL-9	RG9-35	Rat IgG2a
Ab01060	GITR	DTA-1	Rat IgG2a
Ab00374	GITR	YGITR 860.103.5	Rat IgG2b
Ab00631	GITR	YGITR765	Rat IgG2b

Catalog ID	Mouse Target	Antibody Clone	Original Format
Ab00630	GITRL	YGL386	Rat IgG1
Ab01026	HVEM	HMHV-1B18	Hamster IgG
Ab00814	ICOS	7E.17G9	Rat IgG2b
Ab00882	ICOSL	HK5.3	Rat IgG2a
Ab00372	LAG3	YLAG 77.5.1	Rat IgG2b
Ab00110	OX40 (CD134)	OX86	Rat IgG1
Ab00564	OX40L	OX-89	Rat IgG1
Ab00813	PD-1	RMP1-14	Rat IgG2a
Ab01017	PD-1H (VISTA)	mam82	Mouse IgG1
Ab01016	PD-1H (VISTA)	MH5A	Hamster IgG
Ab00373	PD-L1	YDC 127.1.1	Rat IgG2a
Ab00883	PD-L2	TY25	Rat IgG2a
Ab00875	PDPN (MAP-Tag)	PMab-1	Rat IgG2a
Ab00973	TIGIT	4D4	Hamster IgG
Ab01258	TIGIT	1B4	Mouse IgG1
Ab01032	TIM-1	3B3	Rat IgG2a
Ab01110	TIM-2	RMT2-14	Rat IgG2a
Ab01057	TIM-3	2C12	Rat IgG1
Ab01058	TIM-4	5G3	Rat IgG1
Ab01059	TIM-4	3A1	Rat IgG1

## Engineered Fc Fusion Proteins

Catalog ID	Mouse Protein	Ig Fc Domain
Pr00233-1.9	4-1BB Fc-Fusion Protein	Mouse IgG1
Pr00159-1.28	4-1BBL Fc-Fusion Protein	Mouse IgG1
Pr00110-1.9	B7.1/CD80 Fc-Fusion Protein	Mouse IgG1
Pr00158-1.9	B7-H3 Fc-Fusion Protein	Mouse IgG1
Pr00224-1.9	BTLA Fc-Fusion Protein	Mouse IgG1
Pr00231-1.9	CD155 Fc-Fusion Protein	Mouse IgG1
Pr00160-1.9	CD27 Fc-Fusion Protein	Mouse IgG1
Pr00113-1.9	CD47 (C33G) Fc-Fusion Protein	Mouse IgG1
Pr00205	Fc-CD52 Fusion Protein	Mouse IgG1
Pr00206	CD52-Fc Fusion Protein	Mouse IgG1
Pr00161-1.28	CD70 Fc-Fusion Protein	Mouse IgG1
Pr00225-1.9	CD80 Fc-Fusion Protein	Mouse IgG1
Pr00226-1.9	CD86 Fc-Fusion Protein	Mouse IgG1
Pr00230-1.9	CD96 Fc-Fusion Protein	Mouse IgG1
Pr00155-1.9	CTLA4 Fc-Fusion Protein	Mouse IgG1

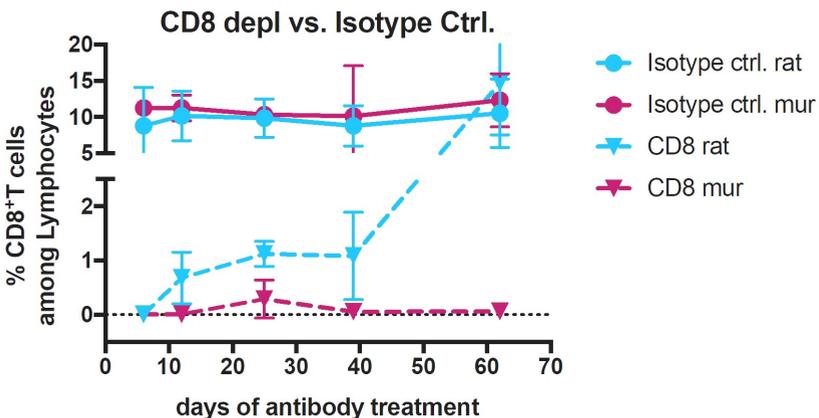
Catalog ID	Mouse Protein	Ig Fc Domain
Pr00223-1.9	HVEM Fc-Fusion Protein	Mouse IgG1
Pr00153-1.9	ICOS Fc-Fusion Protein	Mouse IgG1
Pr00157-1.9	ICOSL Fc-Fusion Protein	Mouse IgG1
Pr00109-1.9	IL2 Fc-Fusion Protein	Mouse IgG1
Pr00162-1.9	Lag3 Fc-Fusion Protein	Mouse IgG1
Pr00154-1.9	OX40 Fc-Fusion Protein	Mouse IgG1
Pr00152-1.9	PD-1 Fc-Fusion Protein	Mouse IgG1
Pr00112-1.9	PDL1 Fc-Fusion Protein	Mouse IgG1
Pr00156-1.9	PDL2 Fc-Fusion Protein	Mouse IgG1
Pr00163-1.9	TIGIT Fc-Fusion Protein	Mouse IgG1
Pr00227-1.9	TIM-1 Fc-Fusion Protein	Mouse IgG1
Pr00228-1.9	TIM-3 Fc-Fusion Protein	Mouse IgG1
Pr00232-1.28	TL1A Fc-Fusion Protein	Mouse IgG1
Pr00111-1.9	TNFR1 Fc-Fusion Protein	Mouse IgG1
Pr00164-1.9	VISTA Fc-Fusion Protein	Mouse IgG1

# Long-Term CD8 T-cell Depletion

Absolute Antibody has engineered mouse-anti-mouse versions of the established anti-CD8 antibodies YTS 169.4, YTS 105.18 and YTS 156.7 for improved performance *in vivo*.

- Reduced immunogenicity as antibody species is matched to host
- No reduction in depletion through anti-drug-antibodies after 60+ days
- Fc Silent formats available as non-depleting blockers and controls
- Multiple clones available for ultra-long treatments

Case Study: Recombinant Anti-CD8 antibody maintains T-cell depletion for over 60 days when reformatted as a mouse IgG2a, whereas the original rat IgG2b format loses efficacy after two weeks.



**Figure:** CD8+T-cell population in mice treated with anti-CD8 antibodies for depletion and isotype controls. Mouse IgG2a format is shown in purple and Rat IgG2b is shown in blue. Injections of 250 µg every 4 days. Unpublished data, courtesy and property of the University of Basel, Switzerland.

# Recombinant RMP1-14

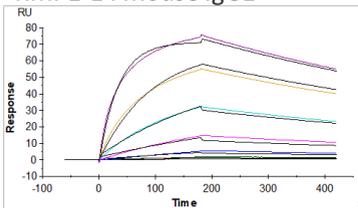
## Mouse-Anti-Mouse PD-1 Antibodies

Absolute Antibody has engineered the popular antibody clone RMP1-14 for improved performance *in vivo*.

- Reduced immunogenicity as antibody species is matched to host
- Choose antibody Fc without affecting binding
- Fc Silent formats available
- Explore new possibilities by combining our antibodies into custom bispecific reagents

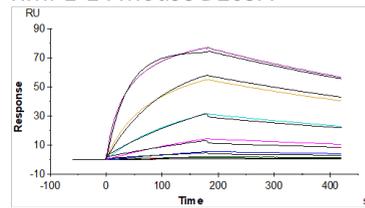
Case Study: Biacore analysis of four recombinant RMP1-14 antibodies shows unchanged anti PD-1 activity in a range of mouse formats.

RMP1-14 Mouse IgG1



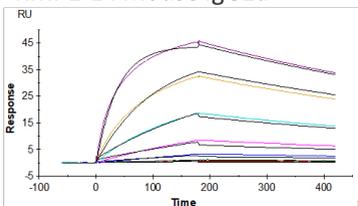
ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)
2.899E+4	0.001304	4.497E-8	77.40

RMP1-14 Mouse D265A



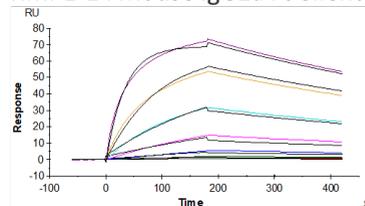
ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)
2.974E+4	0.001291	4.342E-8	74.76

RMP1-14 Mouse IgG2a



ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)
2.674E+4	0.001224	4.576E-8	46.80

RMP1-14 Mouse IgG2a Fc Silent™

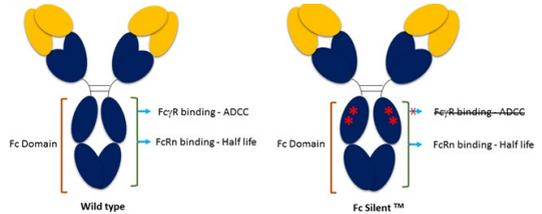


ka (1/Ms)	kd (1/s)	KD (M)	Rmax (RU)
2.697E+4	0.001244	4.611E-8	78.87

# Fc Silent™ Antibodies

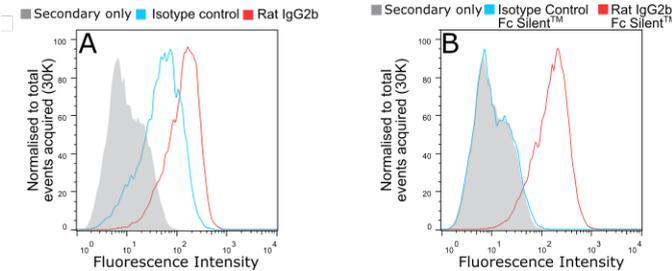
## Turning down the background noise

Specifically designed to reduce non-specific background antibody staining, our Fc Silent™ antibodies can instantly improve experiment quality.

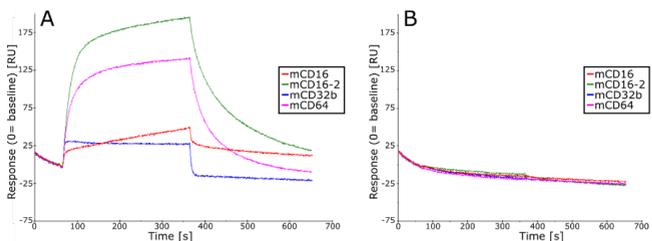


A significant cause of non-specific background staining is the binding of antibodies to Fcγ receptors (FcγRs), making experiments involving FcγR-expressing cells difficult to interpret.

Fc Silent™ antibodies contain engineered Fc domains with abolished FcγR binding. Fc Silent™ does not affect other properties of the antibody, including its compatibility with secondary antibodies.



**Figure 1:** Flow-cytometry of BMDMs stained with wild-type Rat IgG2b (A) and Fc Silent™ (B) anti-F4/80 and isotype control antibodies, followed by an anti-rat secondary antibody. Using Fc Silent™ abolishes non-specific FcγR driven staining, for cleaner and more accurate data.



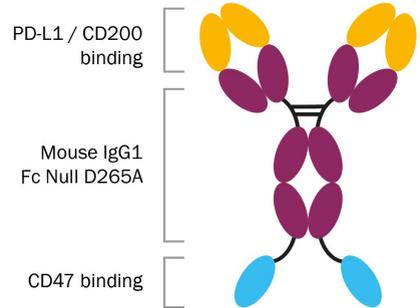
**Figure 2:** Sensogram showing binding of rat wild-type (A) and Fc Silent™ (B) anti-F4/80 antibodies to immobilized murine FcγRs. BIAcore SPR binding analysis shows that the engineered Fc Silent™ antibody has no interaction with FcγRs.

# Exclusive: Murine Bispecific Antibodies

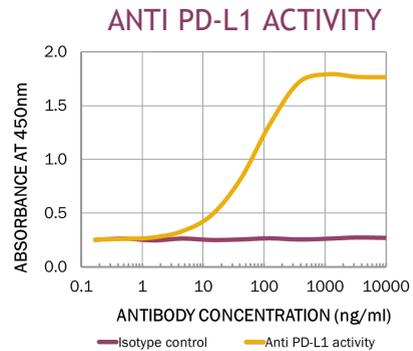
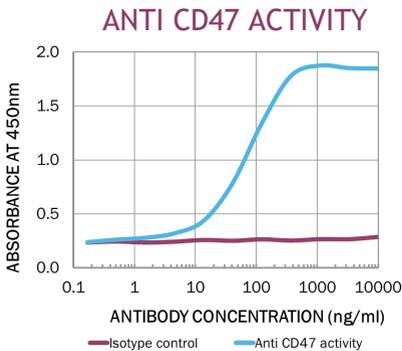
## CD47/PD-L1 CD47/CD200

CD47, CD200 and PD-L1 are high-profile targets in immunotherapy research, as they can help tumours evade both the innate and adaptive immune systems.

Our murine bispecifics allow researchers to target these responses in combination in *in vivo* mouse models.



- Mouse-anti-mouse bispecific antibody with silenced Fc effector function
- Matched to host species for low immunogenicity
- High purity and low endotoxin content for *in vivo* applications
- Defined stoichiometry of binding domains
- ‘Mix-and-match’ from our other targets to build your own bispecific



**Figure:** ELISA data showing binding activity of our CD47/PD-L1 bispecific antibody to murine CD47 (A) and murine PD-L1 (B) relative to an antibody isotype control.

# Research-Grade Biosimilars

Our biosimilars are available with mouse, rabbit, rhesus monkey and cynomolgus monkey constant domains in addition to the original human formats.

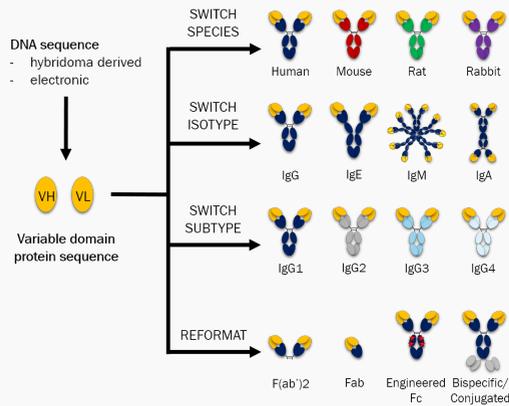
Antibody	Target and Expected Species Reactivity	
Abciximab	CD41	Human
Adalimumab	TNF alpha	Human
Arcitumomab	carcinoembryonic antigen (CEA)	Human
Basiliximab	IL-2R alpha (CD25)	Human; Rhesus Monkey; Cynomolgus Monkey
Bevacizumab	VEGF	Human
Briakinumab	IL-12/23	Human
Campath-1G	CD52	Human; Rhesus Monkey; Cynomolgus Monkey
Campath-1H	CD52	Human; Rhesus Monkey; Cynomolgus Monkey
Campath-6	CD25	Human
Campath-9H	CD4	Human
Cetuximab	EGFR	Human
Clenoliximab	CD4	Human; Chimpanzee
CSL311	CD131	Human
Daclizumab	IL-2R	Human; Rhesus Monkey; Cynomolgus Monkey
Drozitumab	DR5	Human
Eculizumab	C5	Human
Efalizumab	CD11a	Human
EP3-1	CD98	Human
Epratuzumab	CD22	Human; Rhesus Monkey; Cynomolgus Monkey
Felvizumab	RSV	RSV
Galiximab	CD80	Human
Gemtuzumab	CD33	Human
Humicade	TNF alpha	Human
Infliximab	TNF alpha	Human
JOVI.1	V(beta)3 TCR	Human
Matuzumab	EGFR	Human
Mogamulizumab	CCR4	Human
MT310	CD4	Human; Rhesus Monkey; Cynomolgus Monkey
Muromonab	CD3 epsilon	Human
Natalizumab	Integrin alpha 4	Human
Neuradiab	Tenascin	Human
Nimotuzumab	EGFR domain III	Human
Omalizumab	IgE	Human
Oxelumab	OX40L	Human
Pateclizumab	Lymphotoxin alpha	Human
R-125224	Fas	Human
Rituximab	CD20	Human; Rhesus Monkey; Cynomolgus Monkey
Ruplizumab	CD40L	Human
Satumomab	Tumor associated glycoprotein (TAG) 72	Human
TA99	TRP-1, gp75	Human
Tabalumab	CD257 (BAFF)	Human; Cynomolgus Monkey; Rabbit
TES-C21	IgE	Human
Tocilizumab	IL-6R	Human
Trastuzumab	erbB-2 (Her-2/neu)	Human
Volociximab	alpha 5 beta 1 Integrin	Human

# Custom Services Overview

## Antibody Sequencing

Obtain antibody sequence from hybridoma cells or from purified antibody protein.

## Antibody Engineering

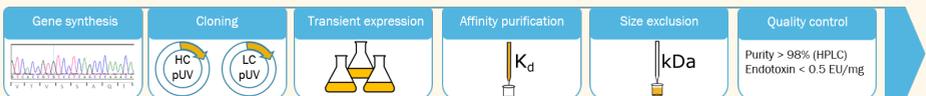


- Choose any antibody species, isotype and subtype
- Antibody fragments, engineered Fc domains, bispecific antibodies
- Addition of functional protein domains
- Humanization

## Recombinant Antibody Expression

Our HEXpress™ transient expression service was launched as a faster and more affordable alternative to stable CHO cell line generation.

- Serum-free mammalian transient expression system (HEK293, CHO on request)
- Express milligram to gram quantities of antibody in as little as 5 weeks
- UK antibody manufacturing facility certified under ISO 9001:2015
- HEXpress™ Silver guarantees > 98 % purity and < 1 EU/mg endotoxin levels
- HEXpress™ Gold is highly customizable to cater for specific requirements

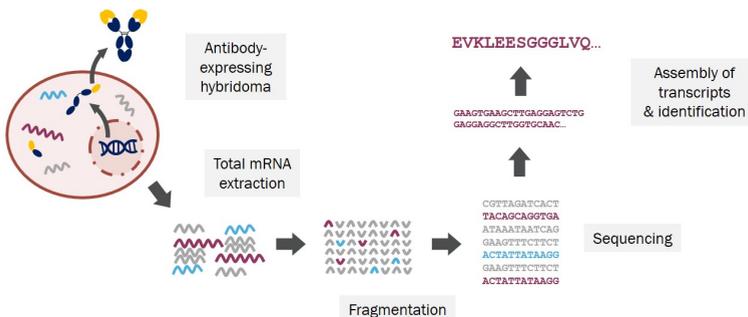


# Hybridoma Sequencing

Hybridomas can take significant time and resources to develop, but are easily lost. Antibody production diminishes over time, often requiring enrichment or re-cloning. Somatic mutations can also impair functionality, and isotype switching occurs naturally. Contamination can happen easily, and liquid nitrogen storage is prone to errors that that can result in the loss of samples. Your monoclonal is valuable, so keep it safe.

In addition, knowing your antibody sequence enables antibody engineering and recombinant production, and allows you to obtain intellectual property rights.

- Sequence any antibody species or isotype
- Identify all variable domains and constant domains present and quantify their relative abundance
- No-sequence-no-fee for any viable hybridoma
- Sequence delivered within 4 weeks
- Use of random primers means that no mutations are introduced

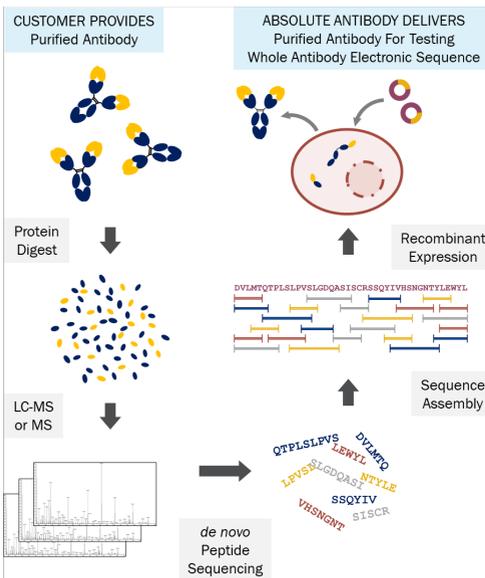


## Our Method

Using a high-throughput (NGS) approach, we sequence all antibody variable domains and constant domains present in your sample. We then deliver a full report characterising your hybridoma's sequences and any heterogeneity or other antibody isotypes present.

# Antibody Protein Sequencing

To complement our hybridoma sequencing service, we can sequence from pure monoclonal antibody for when hybridoma cells are unavailable. By combining mass spectrometry with high-accuracy *de novo* peptide identification and our own transient expression service, we deliver a comprehensive sequencing report and 3 mg of purified antibody in as little as 9 weeks.



- Any species or isotype
- Whole antibody sequencing
- Discrimination between isoleucine and leucine
- Sequence and antibody delivered within 9 weeks
- No-sequence-no-fee

Starting Material	Mass Spectrometric Sequencing	Antibody Expression	Deliverables
<ul style="list-style-type: none"> <li>• Monoclonal antibody</li> <li>• &gt; 0.2 mg</li> <li>• 95% pure</li> </ul>	<ul style="list-style-type: none"> <li>• Enzymatic digest into overlapping peptides</li> <li>• LC-MS/MS using Thermo Orbitrap</li> <li>• <i>de novo</i> peptide identification</li> <li>• Automated peptide assembly</li> <li>• Curation of results by experienced scientists</li> </ul>	<ul style="list-style-type: none"> <li>• Gene synthesis</li> <li>• Cloning</li> <li>• Transient expression in HEK293</li> <li>• Affinity purification</li> <li>• Buffer exchange (PBS)</li> <li>• Sterile filtration</li> </ul>	<ul style="list-style-type: none"> <li>• Comprehensive sequencing report</li> <li>• 3 mg purified antibody</li> <li>• Purity &gt; 98% (SDS-PAGE)</li> <li>• Endotoxin &lt; 1 EU/mg</li> <li>• QC data</li> </ul>

# Engineering Multispecific Antibodies

One of Absolute Antibody's bespoke engineering services is the design and manufacture of multispecific antibody formats.

## Knobs-into-Holes and other approaches to make multispecific antibodies



In the Knobs-into-Holes (KiH) approach two antibody chains are co-expressed recombinantly with mutations to create a “knob” on one and “hole” on the other, such that heterodimer is the predominant product. This is the most widely-recognised method for making bispecific antibodies, however, by making use of Fab fragments and the single chain Fv (scFv) as building blocks, many more options become available.



- The original KiH IgG format uses a common light chain. This is the simplest approach, but often it cannot be used.
- If two different light chains are used they may pair incorrectly causing a loss of binding. Using scFvs avoids this.
- Not all IgGs may be reformatted as an scFv. In these cases, a compromise format could be the solution.



- Another solution is to append another specificity onto an IgG. Typically an scFv is added onto the C-terminus of the heavy chain (left and middle) but adding to the light chain is also possible (right). An scFv could also be added onto the N-terminus, but this might impact binding and require extra considerations (e.g. of linker lengths).
- The Knobs-in-Holes approach can be combined with appended scFvs to make trispecific antibodies and more.



# Antibody Formats We Have Made

All of the antibodies, fragments, and fusion proteins on this page have been made here at Absolute Antibody.

## Isotypes



IgG



IgE



IgM

## Fragments



Fab



scFv



dAb



Free LC



Fc



dAb-Fc



Fab<sub>2</sub>



scFv-Fc



Half Antibody

## Multispecifics



KIH-IgG  
(common LC)



κλ-body  
(common HC)



KIH-Fc-  
Fab/scFv



IgG-scFv



IgG-dAb



scFv-Fc-  
scFv



Trispecific



KIH  
Trispecific



Tandem  
scFv

## Fc Fusion Proteins



Fc Fusion  
(N-terminal)



Fc Fusion  
(C-terminal)



Mono Fc  
Fusion



KIH Fc  
Fusion



KIH Fc  
Fusion



Mono Fc  
Fusion



Bispecific  
Fc Fusion



Bispecific  
Fc Fusion

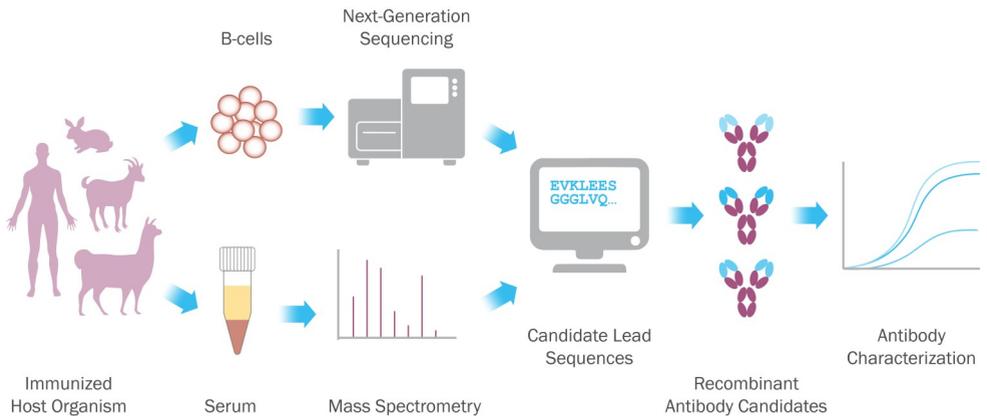


Bispecific  
Fc Fusion

# Immortalizing Polyclonal Antibodies

We now offer polyclonal antibody sequencing, in partnership with Digital Proteomics.

- Produce a polyclonal reagent that is both reproducible and in unlimited supply.
- Harness the native immune system to identify new antibody candidates.



## You Provide:

- Serum and B-cells from your immunized host organism.
- OR
- Purified antigen to use for immunization.
- OR
- Tell us your target, we do the rest.

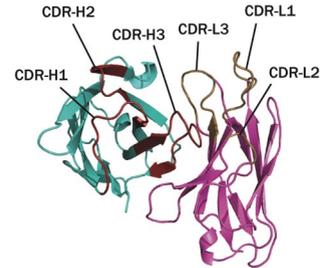
## We Deliver:

- Up to 10 heavy chain sequences and 10 light chain sequences selected by our discovery engine.
- Extended repertoire with proteomic evidence in an interactive report.
- Antibody engineering into any species and isotype upon request.
- Antibody characterization data.
- All 100% royalty free.

# Guaranteed Antibody Humanization

At Absolute Antibody, our investors and scientific advisors have a long history in humanization dating back to the first humanized antibody Campath-1H in 1988.

We deliver a panel of humanized variants from your clone, and guarantee to produce a variant with comparable activity to the parent. Our process also identifies variants with preferential manufacturing criteria (e.g. high expression, low aggregation).



**Figure:** Example ribbon diagram of antibody VH and VL domains.

## Process

- Sequencing if necessary (hybridoma sequencing or *de novo* protein sequencing)
- Modelling of parent VH and VL domains
- Alignment with a panel of preferred human germline sequences
- Analysis of sequence liabilities and selection of de-risked frameworks
- Structure guided CDR grafting onto preferred germline backbones
- A minimum of 12 different humanized sequences generated
- Synthesis, cloning and small scale production of all humanized variants, original parent and chimeric controls
- Early stage manufacturability assessment: measurement of titre and aggregate content

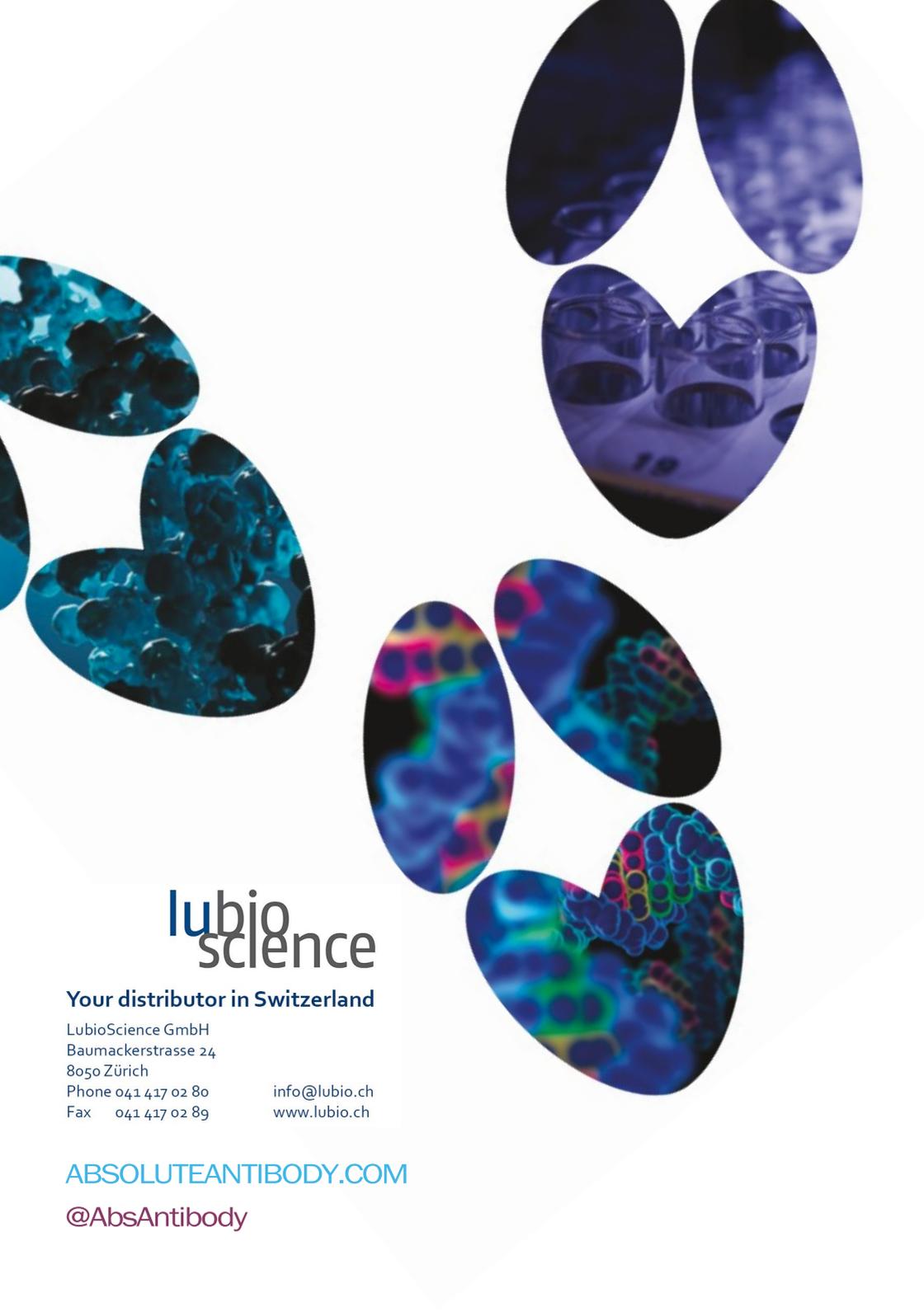
## Timeline

- 8 weeks from receipt of sequence

## Deliverables

- Humanization report detailing humanization strategy, sequences and antibody production analytics (measurement of titre and aggregate content)
- Small scale production (~1mg) of all humanized variants, original parent and chimeric controls

- 100% success rate
- No success, no fee
- Scale up to grams of antibody
- Scale out to 10s of variants
- Royalty-free



# lubio science

**Your distributor in Switzerland**

LubioScience GmbH  
Baumackerstrasse 24  
8050 Zürich  
Phone 041 417 02 80  
Fax 041 417 02 89

[info@lubio.ch](mailto:info@lubio.ch)  
[www.lubio.ch](http://www.lubio.ch)

[ABSOLUTEANTIBODY.COM](http://ABSOLUTEANTIBODY.COM)

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