Choosing the Best HuCAL® Antibody Format

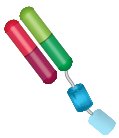
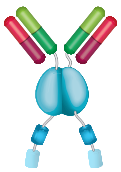
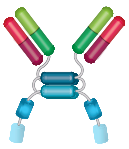
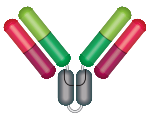
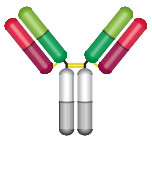
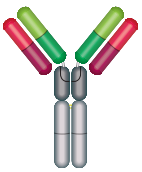
In the detailed discussions during the design of your antibody generation project, you will be asked to choose a Fab format for the first set of antibodies you receive for testing. Our technical specialists will guide you, based on your experimental needs and the intended applications for the antibodies. Options include monovalent or bivalent Fab antibody with a choice of epitope or peptide tags, plus conversion to full-length immunoglobulin, with a choice of isotype. Any antibody can be reordered in one or more alternative formats at any stage after the initial generation project.

Our new TrailBlazer antibody services offer additional special options that give extra versatility for experimental design:

- Site-specific conjugation of selected formats, for improved assay reproducibility
- Fast assembly of different Fab and Ig-like formats, saving 4-6 weeks conversion time

This guide provides details of the full range of antibody formats available, including the new TrailBlazer options, the composition and molecular weights of the epitope tags, and suitability for use in particular applications.

Examples of HuCAL Antibody Formats:

Monovalent Fab		Bivalent Fab			Immunoglobulin	
						
Fab-FH	Fab-FSpy2H SpyTag	Fab-A-V5Sx2	Fab-dHLX-FH	Fab-FH-X22 SpyTag+ BiCatcher	Full length Ig	Ig-like SpyTag+ FcCatcher

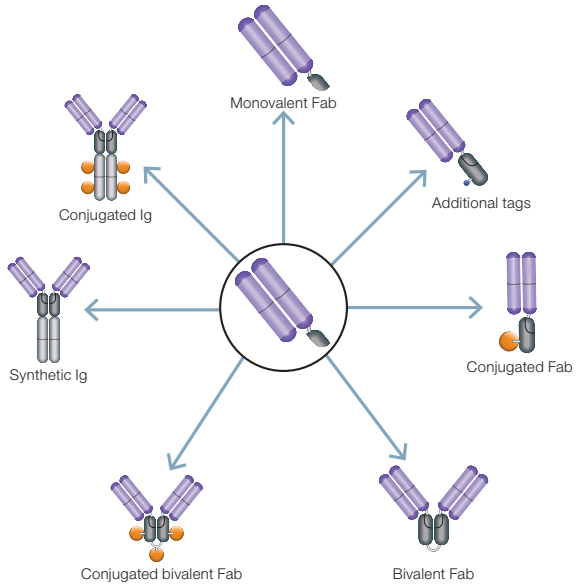
Contents

A detailed description of HuCAL recombinant monoclonal antibody generation services, plus protocols, complementary reagents, and application examples, can be found by visiting **bio-rad-antibodies.com/HuCAL.**

BIO-RAD

1. TrailBlazer Antibody Formats

TrailBlazer antibodies are made using SpyTag-SpyCatcher technology (1, 2). Coupling a single SpyTag antibody to a range of modified SpyCatchers (adapters) enables site-specific conjugation, conversion from monovalent to bivalent Fabs, and the generation of synthetic Ig-like molecules. The protein ligation (coupling) reaction is rapid, quantitative, specific, and exceptionally robust. In the table below, the antibody formats listed in the first two columns (short name, description) are all made via a coupling reaction between the parent monovalent Fab antibody with a SpyTag (Fab-F-Spy2-H) with the parent adapter (a choice of SpyCatcher, BiCatcher or FcCatcher formats). SpyTag2, SpyCatcher2 and SpyCatcher3 are second and third generation versions of the original SpyTag and SpyCatcher, optimized to improve the coupling reaction time from hours to minutes.



More detailed information about TrailBlazer antibody services featuring the SpyTag-SpyCatcher technology can be found at:

bio-rad-antibodies.com/TrailBlazer.

Short Name	Description	Parent Fab	Parent Adapter	Approx MW (kDa)	Code
Monovalent Fab Formats					
Fab-F-Spy2-H	Fab-SpyTag antibody (DYKDDDDK-, Spy2- and His-6-tags)	Fab-F-Spy2-H		54	ad
Fab-FH-X22	Fab-SpyTag coupled to SpyCatcher2	Fab-F-Spy2-H	H-SpyC2	70	oao
Fab-FH-X22-HRP	Fab-SpyTag coupled to HRP conjugated SpyCatcher2	Fab-F-Spy2-H	H-SpyC2-HRP	70 w/o HRP	oap
Fab-FH-X22-bio	Fab-SpyTag coupled to biotin conjugated SpyCatcher2	Fab-F-Spy2-H	H-SpyC2-bio	70 w/o biotin	oab
Fab-FHF3-X22	Fab-SpyTag coupled to SpyCatcher2 with Flag3 tag	Fab-F-Spy2-H	H-SpyC2-F3	73	odo
Bivalent Fab Formats					
Fab2-FH-X22	Fab-SpyTag coupled to BiCatcher2	Fab-F-Spy2-H	H-BiSpyC2	137	pao
Fab2-FH-X22-HRP	Fab-SpyTag coupled to HRP conjugated BiCatcher2	Fab-F-Spy2-H	H-BiSpyC2-HRP	138 w/o HRP	pap
Fab2-FH-X22-bio	Fab-SpyTag coupled to biotin conjugated BiCatcher2	Fab-F-Spy2-H	H-BiSpyC2-bio	138 w/o biotin	pab
Fab2-FH-X22-PE	Fab-SpyTag coupled to RPE conjugated BiCatcher2	Fab-F-Spy2-H	H-BiSpyC2-PE	137 w/o RPE	pad
Fab2-FHF3-X22	Fab-SpyTag coupled to BiCatcher2 with Flag3 tag	Fab-F-Spy2-H	BiSpyC2-F3H	138	pdo
Immunoglobulin-like Formats					
hIgG1-FH-X23	Fab-SpyTag coupled to human IgG1 FcCatcher3	Fab-F-Spy2-H	hIgG1-FcSpyC3	185	rao
hIgG1-FH-X23-HRP	Fab-SpyTag coupled to HRP conjugated human IgG1 FcCatcher3	Fab-F-Spy2-H	hIgG1-FcSpyC3-HRP	185 w/o HRP	rap
hIgG1-FH-X23-bio	Fab-SpyTag coupled to biotin conjugated human IgG1 FcCatcher3	Fab-F-Spy2-H	hIgG1-FcSpyC3-bio	185 w/o biotin	rab
hIgG2-FH-X23	Fab-SpyTag coupled to human IgG2 FcCatcher	Fab-F-Spy2-H	hIgG2-FcSpyC3	185	rdo
hIgG3-FH-X23	Fab-SpyTag coupled to human IgG3 FcCatcher3	Fab-F-Spy2-H	hIgG3-FcSpyC3	196	reo
hIgG4-FH-X23	Fab-SpyTag coupled to human IgG4 FcCatcher3	Fab-F-Spy2-H	hIgG4-FcSpyC3	185	rfo

Short Name	Description	Parent Fab	Parent Adapter	Approx MW (kDa)	Code
hIgG4-Pro-FH-X23	Fab-SpyTag coupled to human IgG4-Pro FcCatcher3 *	Fab-F-Spy2-H	hIgG4-Pro-FcSpyC3	185	rgo
hIgA-FH-X23	Fab-SpyTag coupled to human IgA FcCatcher3	Fab-F-Spy2-H	hIgA-FcSpyC3	188	rho
h/mIgG2a-FH-X23	Fab-SpyTag coupled to mouse IgG2a FcCatcher3	Fab-F-Spy2-H	mIgG2a-FcSpyC3	186	sao
h/rbIgG-FH-X23	Fab-SpyTag coupled to rabbit IgG FcCatcher3	Fab-F-Spy2-H	rbIgG-FcSpyC3	184	sco

* Human IgG4-Pro FcCatcher has a mutation S228P in the core hinge region that prevents the formation of IgG4 half molecules.

2. Fab Antibody Formats and Epitope Tag Combinations

Short Name	Description	Approx MW (kDa)	Fab Clone Code*
Monovalent			
Fab-FSpy2H	Fab antibody (DYKDDDDK-, Spy2- and His-6-tags)	54	ad
Fab-FH	Fab antibody (DYKDDDDK- and His-6-tags)	52	ca
Fab-V5H	Fab antibody (V5- and His-6-tags)	53	cd
Fab-MH	Fab antibody (c-myc- and His-6-tags)	52	cb
Fab-V5Sx2	Fab antibody (V5- and StrepX-StrepX-tags)	56	dc
Fab-FSx2	Fab antibody (DYKDDDDK- and StrepX-StrepX-tags)	54	da
Fab-MSx2	Fab antibody (c-myc- and StrepX-StrepX-tags)	55	db
Fab-H	Fab antibody (His-6-tag)	51	cc
Fab-Sx2	Fab antibody (StrepX-StrepX-tags)	53	de
Fab-CysH	Fab antibody (Cys with His-6-tag)	51	cf
Fab-Cys3H	Fab antibody (three Cys with His-6-tag)	51	ce
Fab-k-ds-H	Disulfide-linked Fab antibody (His-6-tag)	51	ch
Fab-l-ds-H	Disulfide-linked Fab antibody (His-6-tag)	51	ci
Fab-Tc-MH	Fab antibody (Thrombin cleavable, c-myc- and His-6-tags)	53	ck
Bivalent			
Fab-dHLX-FH	Mini-antibody (DYKDDDDK- and His-6-tags)	115	ei
Fab-dHLX-MH	Mini-antibody (c-myc- and His-6-tags)	115	el
Fab-dHLX-H	Mini-antibody (His-6-tag)	112	ek
Fab-dHLX-FSx2	Mini-antibody (DYKDDDDK- and StrepX-StrepX-tags)	119	fe
Fab-dHLX-MSx2	Mini-antibody (c-myc- and StrepX-StrepX-tags)	120	ff
Fab-A-FH	Fab bacterial alkaline phosphatase (BAP) fusion antibody (DYKDDDDK- and His-6-tags)	198	ea
Fab-A-V5H	Fab BAP fusion antibody (V5- and His-6-tags)	200	eb
Fab-A-MH	Fab BAP fusion antibody (c-myc- and His-6-tags)	199	ed
Fab-A-Cys3H	Fab BAP antibody (three Cys with His-6-tag)	197	ef
Fab-A-FSx2	Fab BAP fusion antibody (DYKDDDDK- and StrepX-StrepX-tags)	203	fa
Fab-A-V5Sx2	Fab BAP fusion antibody (V5- and StrepX-StrepX-tags)	205	fb
Fab-A-MSx2	Fab BAP fusion antibody (c-myc- and StrepX-StrepX-tags)	203	fc
Fab-A-H	Fab BAP fusion antibody (His-6-tag)	196	ec
Fab-Max-FH	Fab modified BAP fusion antibody with inactivated enzymatic activity (DYKDDDDK- and His-6-tags)	198	eg
Fab-Max-V5Sx2	Fab modified BAP fusion antibody with inactivated enzymatic activity (V5- and StrepX-StrepX-tags)	205	fd

Note: the HuCAL Fab antibody format does not contain a disulfide bond between light chain and Fd chain; the exception is the Fab-ds-H format.

* From July 1, 2019, HuCAL Fab clone number designation includes a 2- or 3-letter code suffix, denoting the antibody format. Therefore, the same antibody in different formats will have the same numerical number, but different suffixes. Clones generated prior to July 1, 2019 will keep their original clone numbers without the suffix.

3. Tag Sequences and Homodimerization Domains

Short Name	Description	Approx MW (kDa)
Domains		
Fab	Heavy chain variable and first constant domain, and complete light chain	50
dHLX	Synthetic double helix loop helix motif (dimer)	5.2
A	Bacterial alkaline phosphatase (dimer)	47
Max	Modified bacterial alkaline phosphatase with inactivated enzymatic activity (dimer)	47
p53	Domain derived from human p53 (tetramer)	5.8
His-tag Combinations		
H	HHHHHH	0.9
FH	DYKDDDDKGAPHHHHHH	2.1
F-Spy2-H	DYKDDDDKGGSVPTIVMVDAYKRYKGAPHHHHHH	3.9
V5H	GKPIPNPLLGLDSTDAPHHHHHH	2.9
MH	EQKLISEEDLNGAPHHHHHH	2.4
CysH	CHHHHHH	1.1
Cys3H	CCCHHHHHH	1.3
Tc-MH	LVPR↓GSGAPEQKLISEEDLNDAPHHHHHH ↓: Indicates Thrombin cleavage (Tc) site	3.3
Strep-tag Combinations		
S	WSHPQFEK	1.2
FS	DYKDDDDKGAPWSHPQFEK	2.3
FSx2	DYKDDDDKGAPSAWSHPQFEKGGGSGGGSGGSAWSHPQFEK	4.3
V5Sx2	GKPIPNPLLGLDSTDAPSAWSHPQFEKGGGSGGGSGGSAWSHPQFEK	5.6
MSx2	EQKLISEEDLNDAPSAWSHPQFEKGGGSGGGSGGSAWSHPQFEK	4.7

4. Full Length Immunoglobulin Formats

Fab antibodies can be converted to full length human and chimeric human-mouse, human-rat or human-rabbit antibodies when an Fc region is required for the application. The variable heavy and light chain genes are cloned into vectors with the desired constant regions and co-transfected for expression in mammalian cells. Additional isotypes and allotypes are available on request.

- Use Fc region for binding or agglutination reactions
- Evaluate Fc receptor-mediated effects
- Use as a fully human standard e.g. as calibrator and/or control
- In vivo validation of therapeutic antibody interactions in animal model

Description	Ig Clone Code*
Human IgG1 allele G1m3	ia
Human IgG1 allele G1m17, isoallotype nG1m1	im
Human IgG1 allele G1m17,1	il
Human IgG1 allele G1m3,1	in
Human IgG2	ib
Human IgG2/4	io
Human IgG3	ic
Human IgG4	id
Human IgG4-Pro**	ie
Human IgA1	if
Human IgE	ih
Human IgM	ii
Human/Cynomolgus monkey IgG1 chimera (human VH and VL)	kh
Human/Mouse IgG1 chimera (human VH and VL)	ka
Human/Mouse IgG2a chimera (human VH and VL)	kb
Human/Rat IgG1 chimera (human VH and VL)	kc
Human/Rat IgG2a chimera (human VH and VL)	kd
Human/Rat IgG2b chimera (human VH and VL)	ke
Human/Rat IgG2c chimera (human VH and VL)	kf
Human/Rabbit IgG chimera (rabbit CH2 and CH3)	kg

* From June 2019, HuCAL Ig clone number designation includes a 2 or 3 letter code suffix, denoting the antibody format. Therefore, the same antibody in different formats will start with “AbD” followed by the same 5 digit unique identifying number, but will have different suffixes denoting the different formats such as species, isotype and subclass. New productions of clones generated prior to June 2019 will keep the unique identifying AbD number, and be assigned the new 2 or 3 letter suffix, replacing the old format information.

** This antibody format has a mutation S228P in the core hinge region that prevents the formation of IgG4 half molecules.

5. Recommended Fab Antibody Formats by Application

Application	Monovalent	Bivalent	Recommended Secondary Antibodies
Western Blot		√	Anti-human Fab Anti-Strep-tag Anti-His-6 Anti-V5 Anti-BAP Anti-DYKDDDDK Anti-c-myc
ELISA		√	Anti-human Fab Anti-Strep-tag Anti-His-6 Anti-V5 Anti-BAP Anti-DYKDDDDK Anti-c-myc
Immunoprecipitation		√	Anti-human Fab Anti-Strep-tag Anti-His-6 Anti-V5 Anti-DYKDDDDK
Immunohistochemistry		√	Anti-human Fab (for non-human tissue) Anti-Strep-tag Anti-His-6 Anti-DYKDDDDK
Flow Cytometry		√	Anti-human Fab (for non-human tissue) Anti-Strep-tag Anti-DYKDDDDK Anti-His-6
Immunofluorescence		√	Anti-human Fab (for non-human tissue) Anti-Strep-tag Anti-His-6 Anti-V5 Anti-BAP
Affinity Determination	√		
Affinity Chromatography	√		Use a Fab-ds or IgG format to avoid column leakage of the light or heavy chain
Co-crystalization	√		Use Fab-H or Fab TC-MH: use Thrombin to cleave off tags

References

Keeble et al. (2017). Evolving Accelerated Amidation by SpyTag/SpyCatcher to Analyze Membrane Dynamics. Angew Chem Int Ed Engl. 56(52), 16521-16525.

Zakeri et al. (2012). Peptide tag forming a rapid covalent bond to a protein, through engineering a bacterial adhesin. PNAS 109(12),690-697.

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Life Science Group

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