Choosing the Best HuCAL® Antibody Format

A critical step in any Human Combinatorial Antibody Library (HuCAL) generation project is selection of the optimal antibody format.

Options include a monovalent Fab format or a bivalent version, which is functionally equivalent to a typical F(ab')₂ fragment, and choice of epitope or peptide tags which are best-suited to the intended applications.

Many assays benefit from the use of monovalent or bivalent Fab antibodies; certain applications however, require conversion of the Fab antibodies into an immunoglobulin (Ig) format. The epitope tags are an additional feature of the Fab antibodies that can be used for immune-detection in an assay or for immobilization to a matrix. We offer a wide range of common epitope tag Fab antibody formats, all available purified or conjugated to common enzyme and fluorescent labels.

This guide provides a compilation of recommended Fab antibody formats, for use in a range of applications, and details on the composition and molecular weights of the various epitope tags. Please note that Ig formats do not contain any epitope tags.

Contents:

- 1. Recommended Fab antibody formats by application
- 2. Fab antibody formats and epitope tag combinations
- 3. Tag sequences and homodimerization domains
- 4. Full-length immunoglobulin formats



Examples of HuCAL antibody formats:

Fab-FH, monovalent Fab antibody with two epitope tags

Fab-dHLX-FH, bivalent Fab antibody formed via dimerization of dHLX domains (blue cylinders) with two epitope tags

Fab-A-V5Sx2, bivalent Fab antibody formed via dimerization of bacterial alkaline phosphatase (blue modules) with two epitope tags

 $\ensuremath{\text{lgG}}$, a typical full-length immunoglobulin with no epitope tags

Suggested protocols, reagents tested for use with HuCAL antibodies, application examples, and a detailed discussion of our recombinant antibody technology can be found in our HuCAL Antibodies Technical Manual or by visiting **bio-rad-antibodies.com/HuCAL**.



1. Recommended Fab antibody formats by application

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

2.	Fab	antibody	formats	and	epitope	tag	combinations

Short Name	Description	Approx MW (kDa)	Fab Clone Code*
Monovalent			
Fab-FH	Fab antibody (DYKDDDDK- and His-6-tags)	52	са
Fab- <mark>V5</mark> H	Fab antibody (V5- and His-6-tags)	53	cd
Fab-MH	Fab antibody (c-myc- and His-6-tags)	52	cb
Fab- <mark>V5S</mark> x2	Fab antibody (V5- and StrepX-StrepX-tags)	56	dc
Fab-F <mark>S</mark> x2	Fab antibody (DYKDDDDK- and StrepX-StrepX-tags)	54	da
Fab-M <mark>S</mark> x2	Fab antibody (c-myc- and StrepX-StrepX-tags)	55	db
Fab-H	Fab antibody (His-6-tag)	51	сс
Fab- <mark>S</mark> x2	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	се
Fab-k-ds-H	Disulfide-linked Fab antibody (His-6-tag)	51	ch
Fab-I-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-F <mark>S</mark> x2	Mini-antibody (DYKDDDDK- and StrepX-StrepX-tags)	119	fe
Fab-dHLX-M <mark>S</mark> x2	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- <mark>V5</mark> H	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-F <mark>S</mark> x2	Fab BAP fusion antibody (DYKDDDDK- and StrepX-StrepX-tags)	203	fa
Fab-A- <mark>V5S</mark> x2	Fab BAP fusion antibody (V5- and StrepX-StrepX-tags)	205	fb
Fab-A-M <mark>Sx</mark> 2	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- <mark>V5S</mark> x2	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			
А	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					
Н	ННННН	0.9			
FH	DYKDDDDKGAPHHHHHH	2.1			
V5H	GKPIPNPLLGLDSTDAPHHHHHH	2.9			
MH	EQKLISEEDLNGAPHHHHHH	2.4			
CysH	СННННН	1.1			
Cys3H	СССННННН	1.3			
Тс-МН	LVPR↓GSGAPEQKLISEEDLNDAPHHHHHH ↓: Indicates Thrombin cleavage (Tc) site	3.3			
Strep-tag Combinations					
S	WSHPQFEK	1.2			
FS	DYKDDDDKGAPWSHPQFEK	2.3			
F <mark>S</mark> x2	DYKDDDDKGAPSAWSHPQFEKGGGSGGGSGGSAWSHPQFEK	4.3			
V5S×2	GKPIPNPLLGLDSTDAPSAWSHPQFEKGGGSGGGSGGSAWSHPQFEK	5.6			
M <mark>S</mark> ×2	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 models

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 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 in the core hinge region that prevents the formation of IgG4 half molecules.

If you have questions or require additional information on HuCAL antibody formats or would like a complete list of qualified reagents for HuCAL assay development, please contact us. **bio-rad-antibodies.com/HuCAL**

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Life Science Group Web site bio-rad-antibodies.com

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