Fusion BioLabs Ready-to-Use Humanized Bivalent Fab Phage Display Library Information

SKU# ADL-12: Ready-to-Use Humanized Bivalent Fab Phage Display Library Kit

Format: Fab with malE signal peptide-Vk-Cκ (human Igκ)-gD tag: StII signal peptide-VH-CH1 (human IgG1)- Hinge and GCN4 Leucine Zipper)

Diversity: 3.4×10^{10}

The diversity and in-frame of the library was checked by NGS.

Product Description

The Fusion BioLabs Ready-to-Use Humanized Bivalent Fab Phage Display Library Kit is a next-generation antibody discovery platform engineered to bypass the structural and genetic constraints of conventional phage display systems. By combining an ultra-high diversity, synthetic human paratope with a true bivalent structural framework, this kit successfully delivers the high functional performance of a synthetic repertoire alongside the natural avidity advantages of an immunoglobulin molecule.

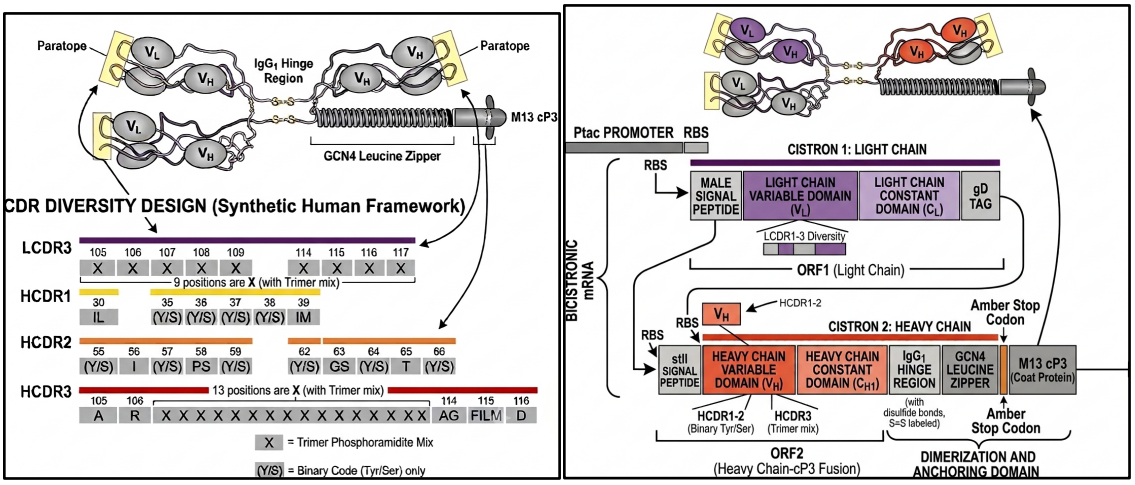
Conventional monovalent platforms often fail to recover low-to-moderate affinity clones, struggle with cell-surface selections, and introduce severe structural mutations when converting single-chain fragments (scFvs) into full IgGs. Fusion BioLabs resolves these roadblocks by engineering a robust bivalent display cassette—integrating a native human IgG1 hinge region and a homodimerizing GCN4 leucine zipper domain directly into the phagemid coat protein vector. The resulting phage particles actively display two functional Fab arms, mimicking natural IgGs to capture powerful avidity effects, drive exceptionally low off-rates, and selectively enrich for receptor-agonistic or cell-internalizing leads right from the panning stage.

Key Repertoire Features

- **Natural IgG Mimicry via True Bivalence:** Incorporates a robust dimerization cassette (IgG1 hinge + GCN4 leucine zipper) between the heavy chain and the cP3 coat protein. This displays dual functional Fab arms per phage particle, driving excellent avidity-based capture on complex targets.
- **Massive, Ultra-High Diversity:** A highly functional synthetic repertoire yielding 3.4×10^{10} unique transformants to maximize sequence space coverage.
- **Light-Chain Driven Reconstitution:** Overcomes the rigid heavy-chain dominance seen in nature by leveraging a heavily diversified 9-amino acid position LCDR3. Given an equal genetic playing field, an engineered LCDR3 readily assumes a dominant role in driving high-affinity antigen recognition.
- **Trimer Phosphoramidite Precision (HCDR3 & LCDR3):** Utilizes controlled trimer codon mixtures within the 13-amino acid HCDR3 and 9-amino acid LCDR3 loops (25% Tyr, 20% Ser, 20% Gly, 10% Ala, and 5% each of Phe, Trp,

His, Pro, Val). This enriches the central paratope with a residue profile optimized for specific, high-affinity protein interfaces.

- **Minimalist Binary Design (HCDR1 & HCDR2):** Solvent-accessible positions within HCDR1 and HCDR2 are restricted to a binary chemical diversity consisting entirely of tyrosine (Tyr) and serine (Ser). This provides clean, highly effective chemical interactions while maintaining excellent baseline loop stability.
- **Structural Framework & Anchor Integrity:** LCDR1 and LCDR2 are intentionally kept unmutated in their native parental sequence because structural data reveals they contribute minimally to the total buried surface area at the antigen interface. Combined with fixed structural anchor boundaries within HCDR3 (preserving native residues at positions 105, 106, 114, and 115), the library maintains outstanding baseline thermal and physical stability.
- **Streamlined IgG Reformatting:** Because selections are performed in a structured, bivalent Fab conformation, discovered leads transfer directly into therapeutic IgG formats without the affinity loss or oligomerization artifacts frequently caused by scFv reformatting.

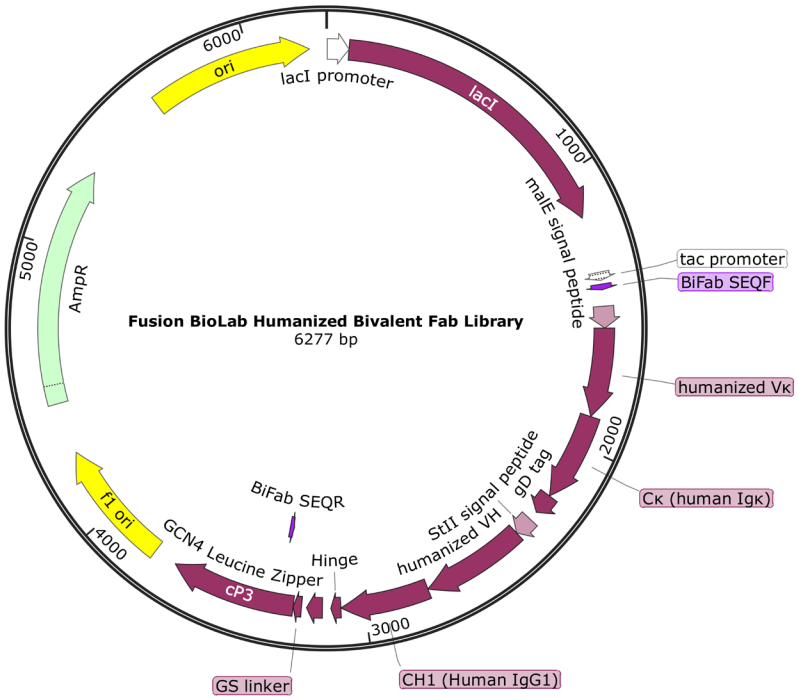


Performance & Selection Advantages

Feature	Monovalent Fab / scFv Libraries	Fusion BioLabs Bivalent Fab Kit
Avidity Benefit	Minimal (Fab) or Uncontrolled Oligomerization (scFv)	**Controlled IgG-like Avidity** (Low Off-Rates)
Cell-Surface Panning	Poor retention of moderate-affinity clones	**Enhanced capture & cell internalization**
Target Flexibility	Stalls on weak or heavily masked epitopes	**High recovery** on low-density or difficult antigens
Biophysical Stability	Variable framework behavior	**Optimized human framework** baseline

Ready-to-Use Humanized Bivalent Fab Library Kit Content

Component	Quantity	Composition
Humanized Bivalent Fab library (ready-to-panning phage; 3.7×10^{12}pfu/ml)	1.0 ml	1×PBS with 25% glycerol
M13KO7 Helper Phage (2×10^{12} pfu/ml)	0.5 ml	1×PBS with 25% glycerol
Chemically Competent TG1 E. coli ($2.5x10^{8}$cfu/ml)	0.5 ml	2xYT with 25% Glycerol
BiFab SEQF Primer (1.6 µM)	0.2 ml	1×TE Buffer
BiFab SEQR Primer (1.6 µM)	0.2 ml	1×TE Buffer



Comments for Fusion BioLabs Vector

Humanized Bivalent Fab (one typical clone)

lac I promoter: bases 5-82
lac I ORF: bases 83-1165
BiFab SEQF priming site: bases 1412-1431
malE signal peptide: bases 1491-1568
gD tag: bases 2211-2285
StII signal peptide: bases 2319-2387
GCN4 leucine zipper: bases 3153-3209

Amber stop codon: bases 3225-3227
cP3 ORF: 3258-3710
BiFab SEQR priming site: 3298-3317
f1 origin: bases 3795-4250
Ampicillin resistance ORF: base 4437-5297
pUC origin: bases 5629-6217

Target Applications

The unique architectural combination of a high-diversity synthetic paratope and a controlled, IgG-like bivalent display format makes this library a powerful engine for demanding discovery campaigns. It is specifically optimized for applications where conventional monovalent Fab or unstable scFv libraries fail to capture or retain functional leads.

1. High-Efficiency Cell-Surface Panning

Isolating therapeutic antibodies against native, multi-pass transmembrane proteins (such as GPCRs, ion channels, and heavily glycosylated tumor-associated antigens) directly on live cells is notoriously difficult.

- **Overcoming Low Epitope Density:** Target receptors are often expressed at low copy numbers on the cell surface. The bivalent display format exploits a controlled **avidity effect**, which significantly drops the effective off-rate of the phage during dense washing steps.
- **Rescuing Moderate-Affinity Hits:** Natural immune repertoires rely on heavy-chain dominance, which often misses valid structural epitopes. By utilizing a heavily diversified LCDR3 to drive antigen binding, this library rescues highly specific, moderate-affinity clones that are normally lost during stringent monovalent selections.
- **Direct Selection of Internalizing Antibodies:** For Antibody-Drug Conjugate (ADC) and payload delivery applications, the bivalent presentation of dual Fab arms effectively mimics natural IgG-mediated receptor engagement. This format triggers active, temperature-dependent receptor-mediated endocytosis, allowing for the direct enrichment of internalizing binders from cell lysates.

2. Phenotypic Functional Agonist Screening

Conventional monovalent phage platforms can only select for binding affinity, meaning functional activation (agonism) must be engineered or screened downstream after reformatting into an IgG.

- **Immediate Receptor Cross-Linking:** Many cell-surface signaling receptors require dimerization or higher-order clustering to trigger downstream intracellular cascades. Because each phage particle displays

two covalently linked, dimeric Fab arms via the engineered IgG1 hinge and leucine zipper, they can actively cross-link adjacent receptors directly during the panning stage.

- **Streamlined Phenotypic Workflows:** Discovered binders can be introduced directly into cell-based functional assays while still in the phage or soluble bivalent format. This completely bypasses the long, unpredictable reformatting steps that frequently result in a structural loss of function or altered binding kinetics when converting scFvs to IgGs.

3. High-Affinity Panning Against Complex and Conserved Targets

- **Conserved and Self-Antigens:** Because the paratope is entirely synthetic and built on a stable human framework, it completely circumvents the immunological tolerance mechanisms of animal-derived hybridoma systems.
- **Engineered Paratope Topography:** Concentrating the trimer phosphoramidite diversity heavily into the elongated HCDR3 (13 positions) and LCDR3 (9 positions)—while maintaining strict framework anchor positions (105, 106, 114, 115)—creates flat, highly complementary binding topologies. This enables specific binding to shallow, hidden, or highly constrained protein interfaces.

Reference:

Lee CV, Sidhu SS, Fuh G. Bivalent antibody phage display mimics natural immunoglobulin. J Immunol Methods. 2004 Jan;284(1-2):119-32.

Persson H, Ye W, Wernimont A, Adams JJ, Koide A, Koide S, Lam R, Sidhu SS. CDR-H3 diversity is not required for antigen recognition by synthetic antibodies. J Mol Biol. 2013 Feb 22;425(4):803-11.