

# THE T-BODY TRISPECIFIC PLATFORM

Invenra's T-Body™ Trispecific Antibody Platform targets three distinct antigens simultaneously, offering a powerful solution for complex diseases by integrating three Fab arms into a single IgG-like structure. This innovative format enhances therapeutic potential through receptor clustering, target-specific engagement, and novel immunomodulatory mechanisms.

## WHY TRISPECIFIC ANTIBODIES?

Trispecific antibodies (tsAbs) represent a next-generation modality that addresses the limitations of monospecific and bispecific antibodies by allowing simultaneous engagement with three unique targets.

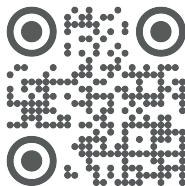
This approach can:

- Increase target specificity
- Reduce antigen escape
- Amplify receptor clustering and internalization
- Enhance immunostimulatory effects

## ENGINEERED FOR ROBUST EXPRESSION AND PURITY

Our T-Body Platform incorporates proprietary CH3 domain pairs engineered for modular, plug-and-play assembly. By substituting CH1/CL with these specialized CH3 domains, we achieve:

- High expression yields in HEK and CHO cells, consistently exceeding 100 µg/mL
- Single-step purification using CH1 resin, delivering >90% purity
- Flexible Fab domain pairing with kappa and lambda light chains
- Significantly reduced misassembly products



## Structure of the T-Body Platform

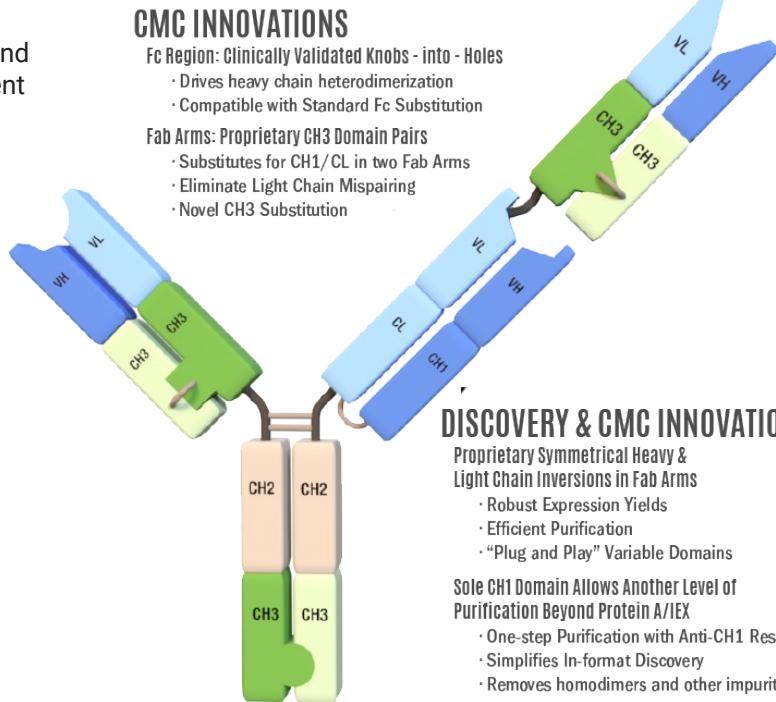
### CMC INNOVATIONS

**Fc Region: Clinically Validated Knobs - into - Holes**

- Drives heavy chain heterodimerization
- Compatible with Standard Fc Substitution

**Fab Arms: Proprietary CH3 Domain Pairs**

- Substitutes for CH1/CL in two Fab Arms
- Eliminate Light Chain Mispairing
- Novel CH3 Substitution



### DISCOVERY & CMC INNOVATIONS

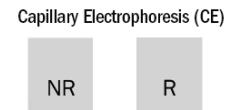
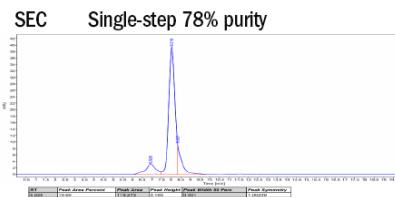
**Proprietary Symmetrical Heavy & Light Chain Inversions in Fab Arms**

- Robust Expression Yields
- Efficient Purification
- "Plug and Play" Variable Domains

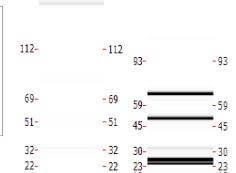
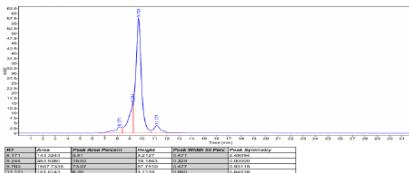
**Sole CH1 Domain Allows Another Level of Purification Beyond Protein A/IEF**

- One-step Purification with Anti-CH1 Resins
- Simplifies In-format Discovery
- Removes homodimers and other impurities

## Example Trispecific Purification



### SMAC



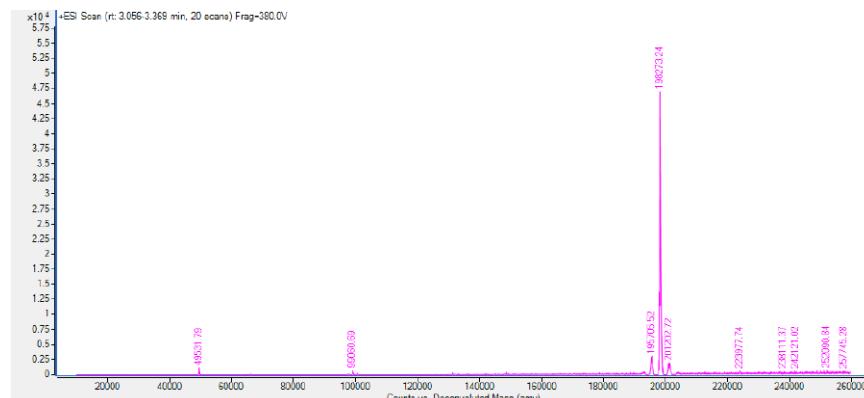
### T-Body Trispecific Expression and Purification

T-Body Trispecifics were produced using single CH1 purification from transient CHO cells, resulting in well-resolved chromatograms.

## OPTIMIZED MANUFACTURING AND CMC

The T-Body Platform is designed to align with standard antibody manufacturing workflows:

- Protein A and Ion Exchange polishing
- Anti-CH1 resin for one-step purification
- Low aggregate formation confirmed by SEC and CE-SDS



Mass Spec Analysis of a T-Body Trispecific

Assembly of the two-step purified T-Body Trispecific was assessed by LC-MS.

## T-BODY BINDING KINETICS

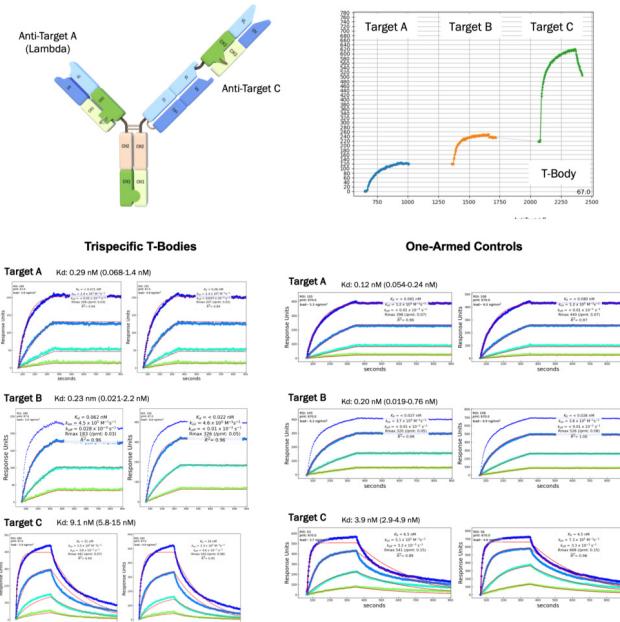
Binding kinetics is crucial in evaluating the therapeutic potential of trispecific antibodies, as it determines the strength, duration, and specificity of antigen-antibody interactions. Our T-Body Platform has been thoroughly investigated with biolayer interferometry (BLI) and surface plasmon resonance (SPR) to assess the simultaneous binding of three distinct antigens. Key kinetic parameters include:

- **Association Rate (ka):** Measures how quickly the antibody binds to the target antigen.
- **Dissociation Rate (kd):** Indicates how rapidly the antibody-antigen complex dissociates.
- **Equilibrium Dissociation Constant (KD):** Reflects the binding affinity, calculated as  $kd/ka$ . Lower KD values indicate stronger binding affinity.

In the T-Body Platform, each Fab arm's binding kinetics are independently assessed to ensure optimal affinity and specificity for each target antigen. This approach provides a comprehensive view of multi-target engagement, enhancing therapeutic efficacy.

## T-BODY PERFORMANCE: SUPERIOR YIELD WITH CHALLENGING ANTIBODIES

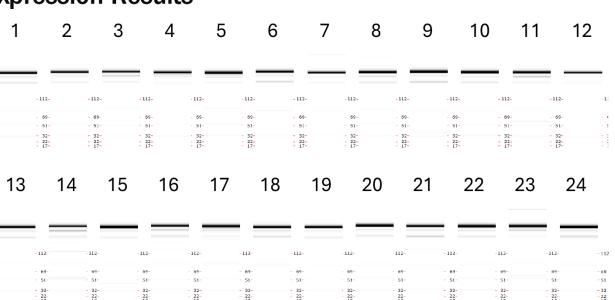
Four clinical antibodies with diverse germlines and developability challenges (Jain et al., PNAS, 2017)—three kappa and one lambda—were combined in a matrixing experiment to generate 24 T-Bodies. The results highlight the platform's robustness, with Expi-CHO expression and one-step CH1 purification delivering strong yields (70–340 mg/L) and high purity (75–95% by non-reducing CE-SDS).



### Clinical Antibody Targets

Name	hVGene	IVGene	Target	HEK Titer (mg/L)	Fab-Tm by DSC (°C)	SGAC-SINS AS100 (NH4)2SO4 (mM)	SMAC Retention Time (Min)	Poly-Specific (P-SHMP Score (0-1)
Atezolizumab (ATE)	IGHV3-23*04	IGHV1-NL1*01	PD-L1	164.1	73.5	300.0	19.3	0.07
Daclizumab (DAC)	IGHV1-4E*01	IGHV1-5*01	CD25	245.1	74.0	900.0	8.8	0.00
Golimumab (GOL)	IGHV3-30*01	IGHV3-11*01	TNFα	163.2	70.0	0.0	12.7	0.23
Guselkumab (GUS)	IGHV5-10-1*04	IGLV1-40*01	IL23	167.3	69.5	700.0	9.2	0.47

### Expression Results



**Matrix Analysis of T-Body Performance (Right)**  
 Expression results from a matrix of clinical antibodies (top table) in the T-Body Platform are shown, bottom. Expi-CHO expression followed by one-step CH1 purification resulted in yields ranging from 70–340 mg/L, with 75–95% purity by non-reducing CE-SDS.