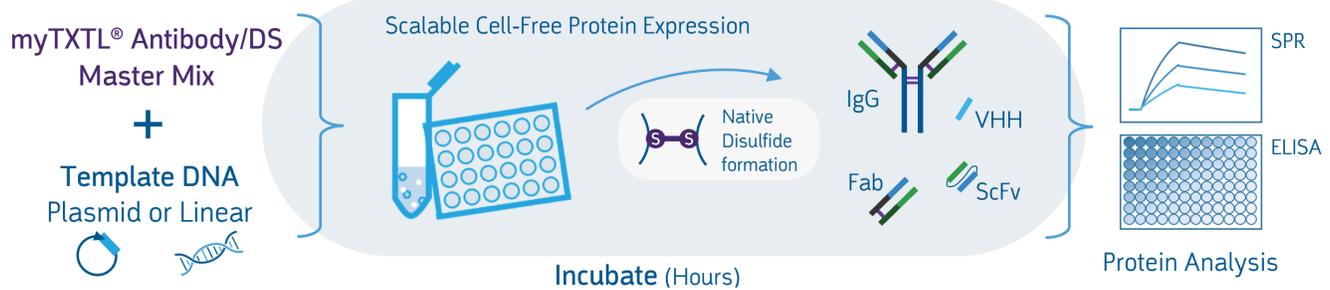


Accelerate Antibody Development with myTXTL[®] Antibody/DS Master Mix



Demand grows for rapid, cost-effective antibody discovery workflows that complement or replace traditional mammalian cell expression systems that have high costs, long development timelines, and limited suitability for quick prototyping. Cell-free protein expression is a powerful alternative, yet many platforms still struggle with protein yield and the proper folding and assembly required for antibodies. The myTXTL[®] Antibody/DS Master Mix from Daicel Arbor Biosciences closes this gap with a ready to use, high performance cell-free solution designed for antibodies and other complex proteins with disulfide bonds. **By integrating an optimized oxidized environment into a single, complete mix, the Antibody/DS Master Mix enables fast, robust, and accessible expression** – accelerating discovery and pushing beyond the constraints of conventional *in vivo* systems.

Disulfide bond formation with ease

The myTXTL Antibody/DS Master Mix (Ab/DS MM) incorporates an optimized oxidative folding environment, proprietary *E. coli* extract preparation, and a formulation tuned for multi-chain assembly. These features enable robust expression of VHHs, ScFvs, Fabs, and full-length IgGs (Figure 1). A comparison of non-reduced and reduced SDS-PAGE samples reveals the oligomeric state of non-reduced antibodies and disulfide bond formation. As expected, non-reduced IgG in Figure 1 migrates as ~160 kDa and Fab migrates as ~50 kDa. Reduction of the DS bonds results in free light and heavy chains, which co-migrate for Fab. Reduction of VHH and ScFv only causes small shifts in gel migration.

Obtaining antibodies has never been faster! Purified full-length IgG products are visible by SDS-PAGE with as little as 3 hours of expression (Fig. 2A). Expression kinetics for IgGs Trastuzumab and Rituximab show rapid accumulation of products that increase in yield for 24+ hours. As shown in Figure 2A, different IgG may require different reaction times to achieve their maximum yield.

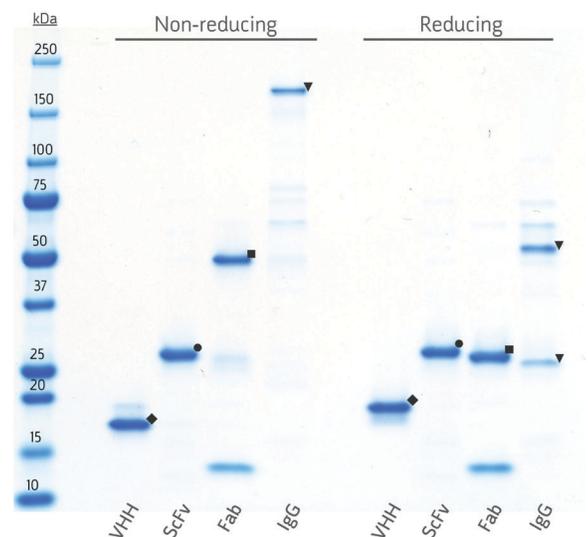


Figure 1 – Antibody formation in myTXTL Ab/DS MM.

Antibodies of varying class were expressed with myTXTL and purified by His-tag (VHH), Protein A (ScFv, IgG), and Strep-tag (Fab). Non-reducing and reducing SDS-PAGE samples were prepared from each eluate. Proteins were visualized by Coomassie staining. Dark grey symbols mark the bands pertaining to each antibody.

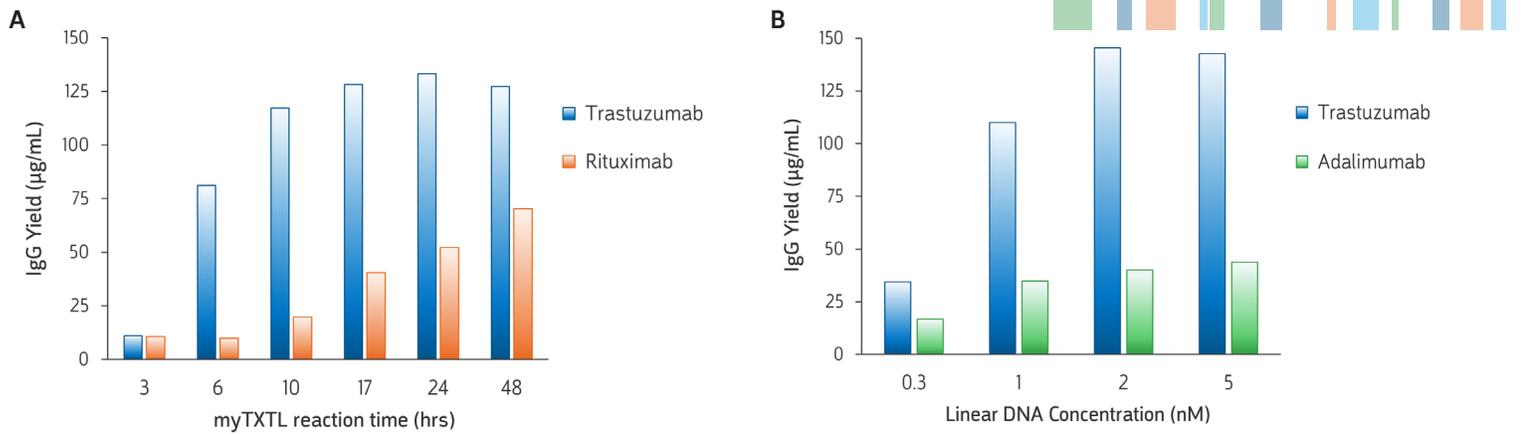


Figure 2 – Effect of reaction time and template DNA concentration on IgG expression. (A) Trastuzumab and Rituximab IgG were expressed for 3 – 48 hours and SDS-PAGE samples were prepared at the designated timepoints. (B) Trastuzumab and Adalimumab IgG were expressed from Linear DNA at concentrations of 0.3 nM – 5 nM per chain and SDS-PAGE samples were prepared after 24-hr expression. In-gel BSA standards were used to determine IgG concentration and yield by densitometry.

myTXTL Ab/DS MM enables the use of linear DNA templates, which are cheaper and faster to synthesize than plasmids. Linear DNA can be quickly generated by PCR or ordered from service providers. Refer to the myTXTL manual for DNA design guidelines. Adalimumab and Trastuzumab IgG expression are detectable by SDS-PAGE with Coomassie staining using only 0.3–5 nM linear DNA per chain (Figure 2B). When expressing antibody from plasmid, 5 nM template DNA per chain is recommended as a starting point. Most IgG expressed with myTXTL Ab/DS MM show optimum yields when expressed at 27°C with template DNA for each chain at a 1:1 nM ratio. Some antibodies may perform better at lower temperatures (16°-20°C) or with a different template DNA ratio (2:1 LC:HC for example) due to differences in antibody sequences and the resulting properties.

Antibody screening: iterate rapidly

The Antibody/DS Master Mix simplifies antibody lead screening by delivering directly assayable antibody products. No cells, no lysis! Many biophysical assays can be conducted using unpurified myTXTL samples. Binding can be immediately assayed by antigen pull-down or ELISA. Add antigen directly to the myTXTL reaction and pull down the antibody to verify binding (Figure 3). Quickly screen lead antibodies by comparing point ELISA results. Using immobilized antigens on a plate, 5 different IgGs, a Fab, and an ScFv show informative ELISA signals when myTXTL reactions are diluted 1,000x – 9,000x (Figure 4).

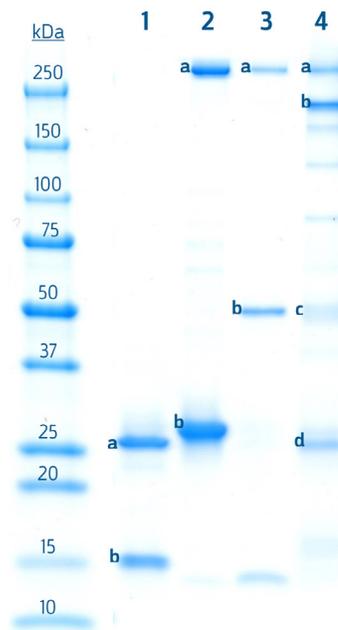


Figure 3 – Antigen Pull-down from myTXTL reactions. Antibodies were expressed in Ab/DS MM. Recombinant antigens were added to dilute reactions and the antibody:antigen complexes were pulled down by capturing antibody on magnetic beads. Antigens are denoted by letter 'a' with GFP in lane 1 and HER2 in lanes 2-4. Antibodies are denoted by letter 'b' with anti-GFP VHH in lane 1, and Trastuzumab ScFv, Fab, IgG in lanes 2, 3, and 4, respectively. Free IgG HC and LC are denoted by 'c' and 'd' in lane 4. Proteins were visualized by Coomassie staining.

When comparing antibodies against a single antigen, the greatest ELISA signals should be generated by antibodies with the best expression, solubility, and antigen binding properties. In Figure 4, three forms of Trastuzumab are compared with the simple ScFv generating the most robust signals due to its high expression and solubility, while binding affinities are similar among the antibody forms (Table 1 and Fig. 7). For more detailed binding information, assays involving surface-capture, like SPR and BLI can be employed with dilute myTXTL reactions. Rapid antibody expression and evaluation with myTXTL accelerates design-and-test cycle iterations, outpaces traditional workflows, and drives discovery.

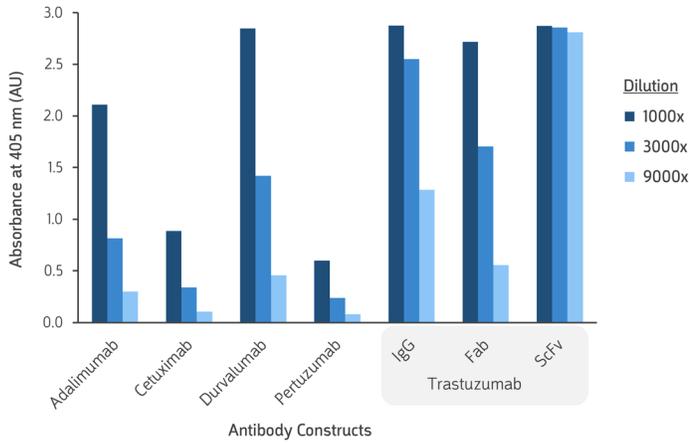


Figure 4 – ELISA signals of antibodies in endpoint myTXTL Ab/DS MM reactions.

To prepare ELISA samples, endpoint myTXTL reactions were diluted 1000-fold to 9000-fold using TBST + 0.2% BSA. The ELISA plate was coated with recombinant antigens overnight. His-tagged Antibodies were detected using an anti-histidine, alkaline phosphatase conjugated secondary antibody.

Purification-ready antibodies

Antibodies can be rapidly purified from Antibody/DS Master Mix using magnetic beads with common purification schemes. For automated purification, myTXTL reactions can be run in multi-well plates compatible with the chosen automation system. Protocols for His-tag, Strep-tag, and Protein A purification from myTXTL are developed and capable of delivering similar antibody yields. A representative antibody panel is shown in Figure 5A following Protein A purification with Gentle Buffers (Thermo Scientific). As displayed in Figure 1, strep-tag purification will co-elute a 12 kDa endonuclease V inhibitor that should not interfere with most assays.

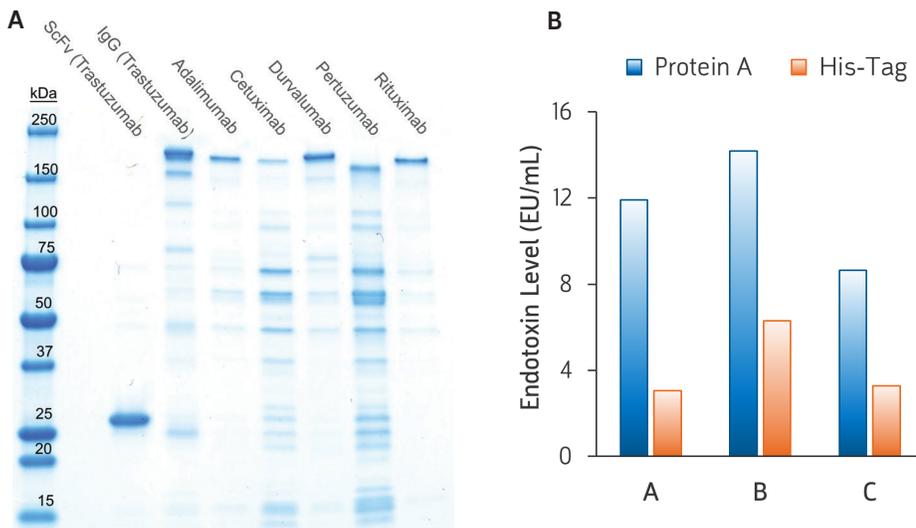


Figure 5 – Antibody Purification and endotoxin removal. (A) Antibodies visualized by SDS-PAGE following Protein A magnetic bead purification and buffer exchange to TBS. (B) Endotoxin level of Trastuzumab IgG samples following purification from myTXTL by His-tag and Protein A with an endotoxin removal wash. Endotoxin quantification was conducted with recombinant factor C (Acro Biosystems) and did not use horseshoe crab products.

For sensitive downstream applications, an endotoxin removal protocol was developed for His-tag and Protein A purification to leave antibody stocks with about 5 – 15 EU/mL (Figure 5B). To deplete endotoxin, a simple wash step using Triton X-114 is added to the antibody purification protocol.

Antibody characterization

Characterization following antibody purification demonstrates that antibodies produced using myTXTL Antibody/DS Master Mix are highly active. Multipoint ELISA can be leveraged to readily obtain EC50 values without expensive equipment. For ELISA-based EC50 determination, purified antibodies were first run on SDS-PAGE gels (Figure 5A) and in-gel BSA standards were used for antibody concentration determination by densitometry. ELISA plates were coated with recombinant antigens (Sino Biological) and an anti-histidine, alkaline phosphatase conjugated secondary antibody was applied to detect His-tagged antibodies. Each myTXTL-produced antibody was diluted across one row of a 96-well plate and EC50 values were determined by curve fitting with a four-parameter logistic function (Figure 6, Table 1). As summarized in Table 1, the determined EC50 values using myTXTL Ab/DS MM are in close agreement with published affinities. This alignment between measured and published affinities underscores the ability of Ab/DS MM to produce biologically relevant, high-performing antibodies.

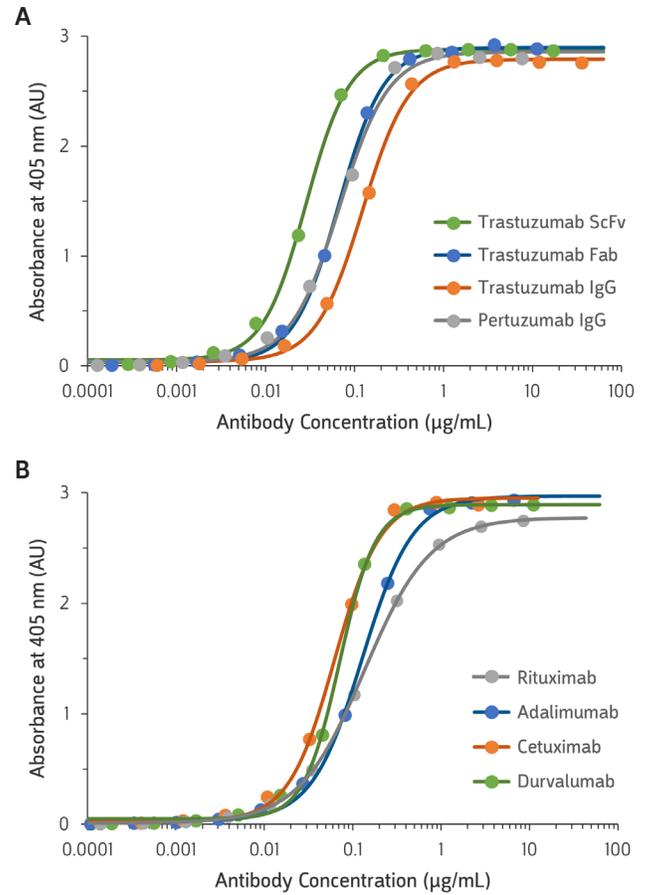


Figure 6 – Multipoint ELISA with myTXTL produced antibodies. Purified antibodies were diluted 3-fold from well-to-well across 11 wells of a 96-well plate. Curve fitting for EC50 determination was done with JMP software.

Antibody Information		Antigen	myTXTL Yield (µg/mL)	ELISA EC50 (nM)	Published Affinity (nM)	Parameter Determined	Publication Source (PMID)
Construct	Class						
VHH_GFP4	VHH	GFP	49	3.4	1.4	Kd	20945358
anti-Fab	VHH	Fab	267	6.8	-	-	-
ScFvT84.66	ScFv	CEACAM5	11	2.4	-	-	-
Trastuzumab	ScFv	HER2	574	1.0	0.3	Kd	38923711
Trastuzumab	Fab	HER2	75	1.3	0.459	EC50	27043557
Adalimumab	IgG	TNF-α	45	0.87	2.81	EC50	30468855
Cetuximab	IgG	hEGFR	5.9	0.42	0.1	EC50	17970081
Durvalumab	IgG	PD-L1	74	0.48	0.831	Kd	33397194
Pertuzumab	IgG	HER2	17	0.46	1.61	Kd	29335579
Rituximab	IgG	CD20	54	0.92	1.9	Apparent Kd	33010205
Trastuzumab	IgG	HER2	239	0.79	0.228	EC50	27043557

Table 1 – Characterization of antibodies produced with myTXTL. Antibody yield was determined by gel densitometry.

Detailed binding analysis of myTXTL-produced antibodies was conducted by an independent researcher who expressed, purified, and performed SPR with two ScFv and two IgG antibodies (Figure 7). SPR experiments were run on a Biacore 8000 (Cytiva) using an NTA sensor chip for His-capture. Anti-HER2 Trastuzumab IgG and ScFv were tested alongside an anti-MBP IgG and ScFv. Following bead purification and buffer exchange, His-tagged antibodies were immobilized on the SPR chip and analyte was injected at varying concentrations. The determined kinetic parameters and dissociation constants are displayed in Figure 7. myTXTL Trastuzumab IgG results are comparable to published values from Trastuzumab BLI measurements ($K_d = 2.77$ nM, $k_{on} = 1.11 \times 10^5$ M⁻¹s⁻¹, $k_{off} = 3.07 \times 10^{-4}$ s⁻¹) using IgG produced with human cells (PMID: 29335579). The comparable kinetic profiles demonstrate that Antibody/DS Master Mix provides functional antibodies that perform as well as industry standard counterparts.

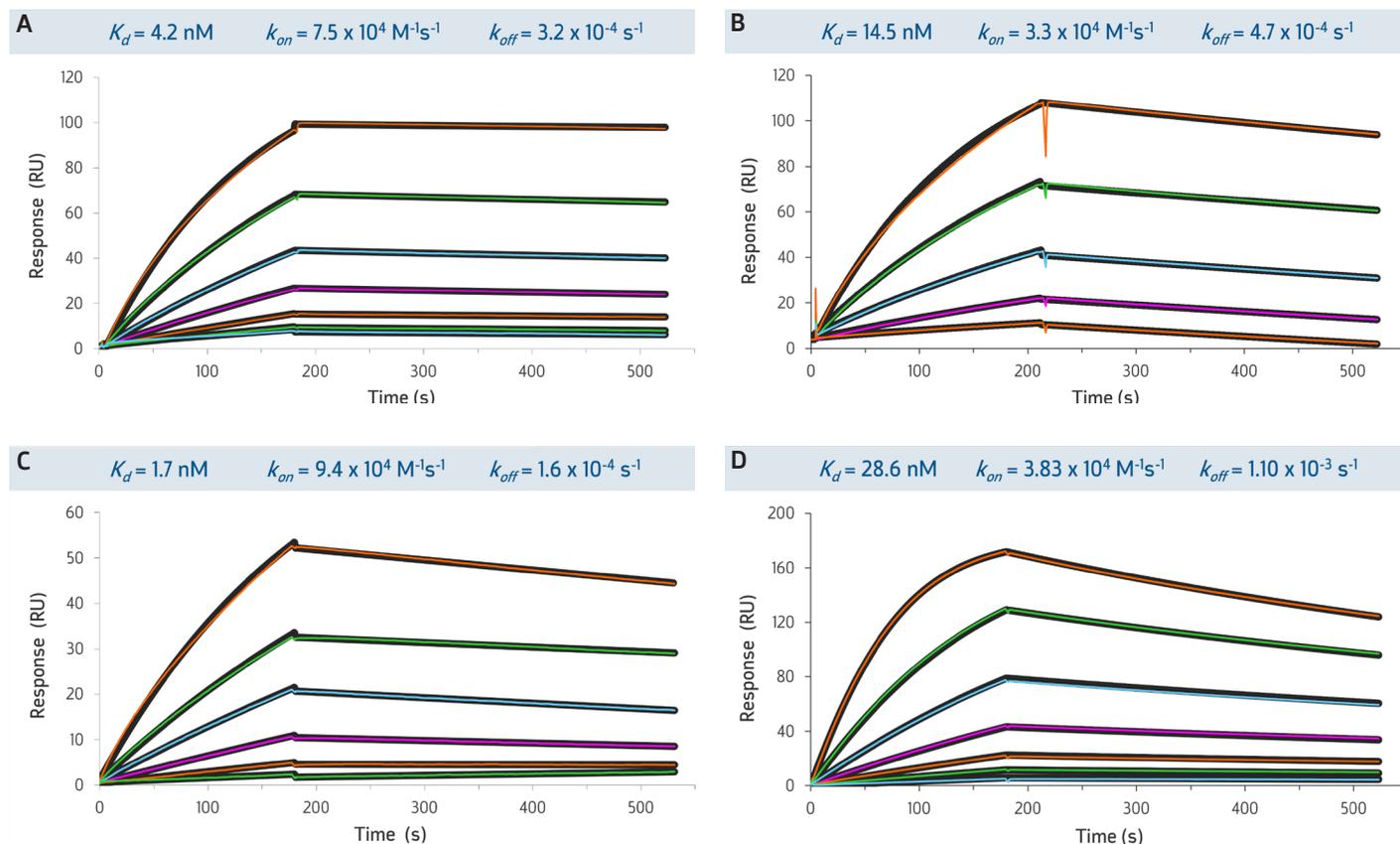


Figure 7 – Binding analysis of antibodies produced with myTXTL. (A) Trastuzumab IgG, (B) Trastuzumab ScFv, (C) anti-MBP IgG, and (D) anti-MBP ScFv were expressed using myTXTL Antibody/DS Master Mix by an independent researcher. The Antibodies were captured on a NTA sensor chip and SPR conducted with a Biacore 8000. Antigens HER2 or MBP were injected at varying concentrations. Data traces are represented by colored lines while fit lines are displayed as black. The kinetic and equilibrium binding parameters are displayed above the corresponding SPR sensorgram. Data collected and analyzed by Dr. Somnath Mukherjee, Kossiakoff Lab, University of Chicago.

myTXTL Antibody/DS Master Mix benefits

- Industry-leading antibody yields, just add DNA!
- Minimal DNA input needed.
- Custom vector not required, supports T7 and any *E. coli* promoter.
- Native support for linear DNA templates and plasmid.
- No expensive instrumentation required.
- End products can be directly assessed for binding/activity (optional purification).
- Kits include a positive control plasmid to evaluate expression and DS bond formation.
- Kit sizes built to match your throughput needs.

Antibody/DS Master Mix
delivers industry-leading
yields!

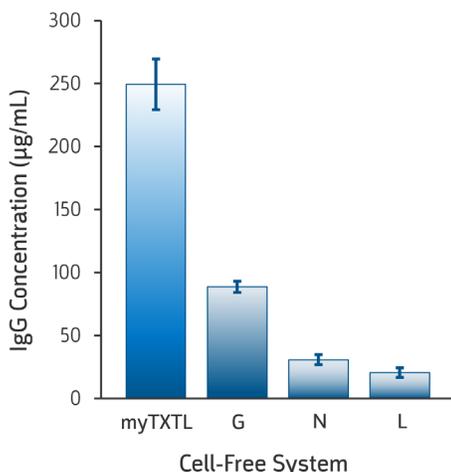


Figure 8 – Trastuzumab expression in myTXTL and alternative cell-free systems. Trastuzumab was expressed in each system following the manufacturers' protocols for IgG or protein with DS bonds. IgG yield was quantified from dilute endpoint reactions using a Trastuzumab ELISA kit (IBL America).

Method: antibody expression with myTXTL Antibody/DS Master Mix

Antibody/DS Master Mix, DS Helper Plasmid, and template DNA were thawed at room temperature, then placed on ice. Expression was typically conducted using 20 µL myTXTL reactions as follows:

VHH, ScFv, Fab Reactions	
Component	Volume (µL)
Antibody/DS Master Mix	15.0
DS Helper Plasmid	0.83
24 nM Template DNA	4.17
Reaction Volume	20.0

IgG Reactions	
Component	Volume (µL)
Antibody/DS Master Mix	15.0
DS Helper Plasmid	0.83
48 nM nM LC DNA	2.08
48 nM HC DNA	2.08
Reaction Volume	20.0

Reactions were incubated in conical 2.0-mL tubes at 27°C. Note that Fab expression was conducted using a single template DNA encoding both LC and HC under one operon. Following expression, reactions were placed on ice for at least 5 minutes and then centrifuged at 16,000 xg for 3 minutes to pellet insoluble material. The soluble reaction material is then ready for downstream assays or purification. For further reaction guidelines, please refer to the myTXTL Antibody/DS Expression Kit Manual.

Links to purification protocols

- His-tag Purification (optional endotoxin removal)
- Protein A Purification (optional endotoxin removal)
- Strep-tag Purification

myTXTL Antibody/DS Expression Kit Product Offerings	
Kit Size	Catalog Number
300 µL	560300
1000 µL	561000
5 mL	5605ML
10 mL	5610ML

Kits include Master Mix, DS Helper Plasmid, and T7 GLuc Control Plasmid. Kit size reflects the Master Mix volume.



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