myTXTL Cell-Free Expression of Antibodies and Antibody Fragments

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Abstract

Antibody discovery and screening processes are time, labor, and cost intensive primarily due to challenging protein expression. A novel myTXTL® Antibody/DS Master Mix enables rapid, cell-free expression of antibody constructs to greatly reduce overall time and resources required for antibody discovery. Historically, cell-free protein expression systems suffered from low yields, inconsistent batch quality and an inability to synthesize active antibody constructs due to a reducing environment. We demonstrate the lot-to-lot consistency of the myTXTL Antibody/DS Master Mix for the expression of a functional disulfide bond containing enzyme. Then, we demonstrate that myTXTL Antibody/DS Master Mix outperforms several existing cell-free systems on the market for both IgG and Fab expression. Due to the cell-free environment of myTXTL, dilutions of endpoint reactions can be used directly in ELISA assays for rapid antibody evaluation. An ELISA of Trastuzumab IgG expressed using several cell-free systems revealed that myTXTL Antibody/DS Master Mix produced 3-fold more active IgG than the nearest competitor. Omitting *in vivo* expression and consistently delivering industry-leading yields enables myTXTL Antibody/DS Master Mix to accelerate antibody screening and evaluation workflows.

Methods

GLucDura, Trastuzumab IgG and Fab fragment were expressed from plasmids containing a T7 promoter. Target proteins were purified using either MagStrep beads (IBA Lifesciences) or Protein A beads (GenScript) . Trastuzumab IgG ELISA was conducted with an ELISA kit from IBL America (Cat. No. TM09013). Cell-free reaction parameters are summarized in Table 1. myTXTL reactions contained target protein plasmid(s), a helper plasmid expressing T7 RNA polymerase, and myTXTL Antibody/DS Master Mix. Competitor kit reactions were setup according to manufacturer's recommendations. Reaction setup and incubation conditions are summarized in Table 1.

Table 1: Cell-free reaction setup

Cell-Free System	# Components per reaction	Reaction Volume (uL)	Reaction Time (hours)	Reaction Temperature (°C)	Shaking Speed (RPM)
myTXTL	3	12	16	27	0
G	9	12	24	37	0
N	7	50	24	25	1000
L	2	50	48	25	700

Lot-to-Lot Consistency of myTXTL Antibody/DS Master Mix

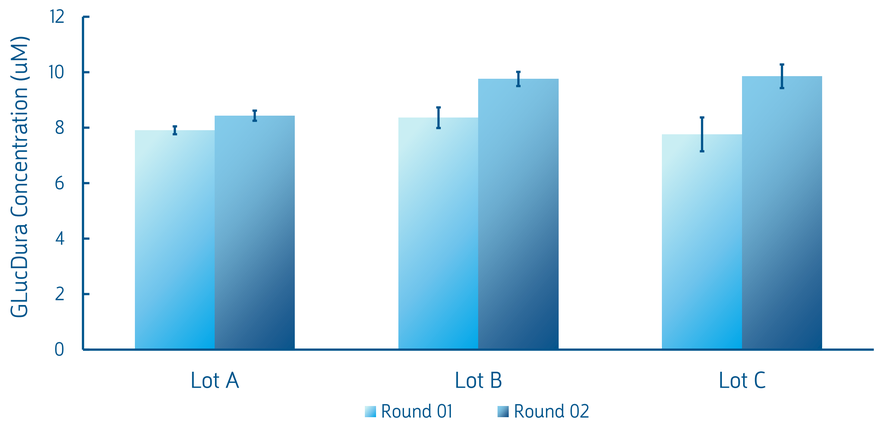
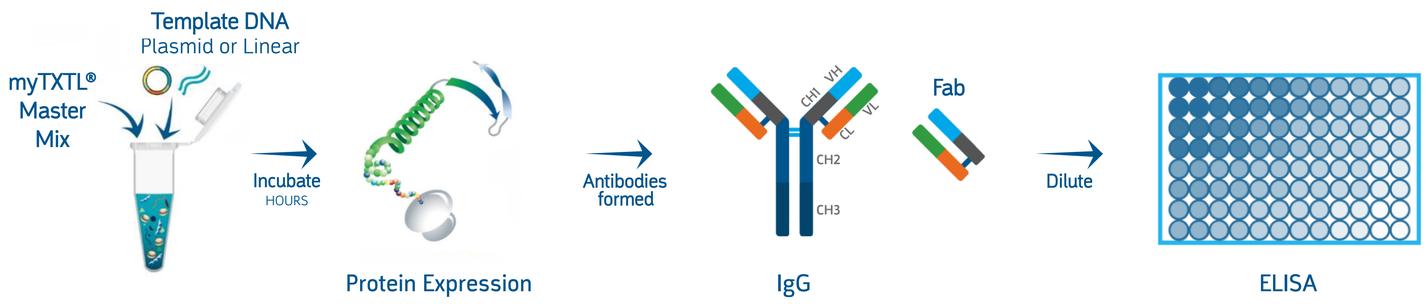


Figure 1. GLucDura expression with Antibody/DS Master Mix: Three independent lots of myTXTL Antibody/DS Master Mix were evaluated for expression of active *Gaussia*-Dura luciferase (GLucDura) using a luminescent activity assay (NanoLight). Expression was tested in two independent rounds.

- All 3 lots of Antibody/DS Master Mix yielded significant and consistent amounts of active GLucDura enzyme, a protein with 5 disulfide bonds.

myTXTL Cell-Free Expression Antibody/DS Workflow



Cell-Free Expression and Evaluation of Trastuzumab Fab

Trastuzumab Fab was expressed in myTXTL Antibody/DS Master Mix and competitor kits with light and heavy chains under a single operon. Expressed Fab was incubated with HER2 antigen and pulled down by a Twin-Strep-tag® on the heavy chain to demonstrate antigen binding.

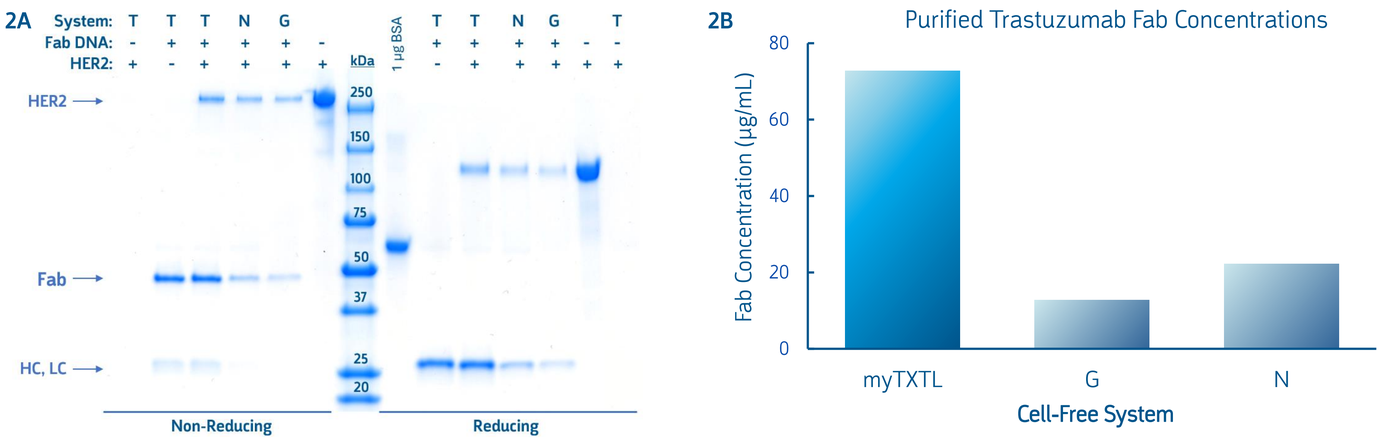


Figure 2. Evaluating cell-free expression of Trastuzumab Fab: SDS-PAGE visualization of pulldown eluates under non-reducing and reducing conditions reveals antigen and native Fab in all cell-free systems tested (**Fig. 2A**). Concentrations of Fab were determined from SDS-PAGE bands using a BSA loading control (**Fig. 2B**).

- myTXTL expressed high yields of Fab, more than competitors N and G (3-fold and 6-fold) and pulled down more HER2 antigen
- Bead purified Fab was of high purity for all kits tested

Twin-Strep-Tag® is a registered trademark of IBA Lifesciences

ELISA and Protein A Purification of Cell-Free Expressed Trastuzumab IgG

Trastuzumab IgG was expressed from two separate plasmids at a 1:1 ratio (HC:LC) in myTXTL Antibody/DS Master Mix for 24 hours and in 3 competitor kits. Duplicate myTXTL and competitor endpoint reactions were diluted and used immediately for an anti-HER2 ELISA.

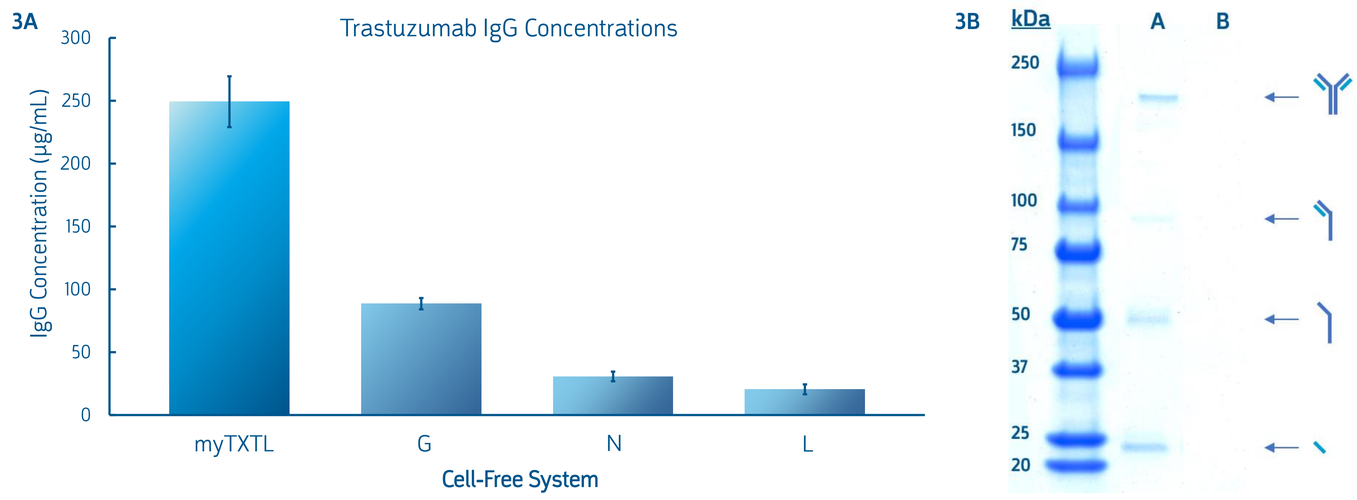


Figure 3. Evaluating cell-free expression of Trastuzumab IgG. Four dilutions of each reaction were evaluated by ELISA to determine average IgG concentrations in endpoint reactions (**Fig. 3A**). Protein A magnetic bead purification of Trastuzumab IgG expressed using myTXTL Antibody/DS Master Mix shows that the 154 kDa IgG band is an abundant product with smaller species also evident on the gel (**Fig 3B**).

- myTXTL expressed high yields of functional IgG, 3x to 11x more IgG per unit volume than competitors
- IgG expressed in myTXTL can be purified with Protein A beads

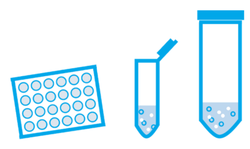
Conclusions

- myTXTL Cell-Free Expression Antibody/DS Master Mix has **robust lot-to-lot performance** for expression of proteins bearing disulfide bonds.
- The Antibody/DS Master Mix **expressed high yields of functional Fab and IgG**, outperforming competitors.
- Use of the myTXTL cell-free expression system can reduce the cost and time of antibody discovery workflows by enabling **antibody expression and evaluation within 24 hours.**
- Want to learn more about cell-free protein expression? Visit our **Booth #711**!

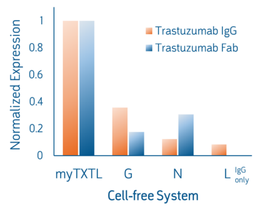
myTXTL Antibody/DS Master Mix Features:

Template DNA
Plasmid or Linear

Reaction Volume
Scalable



Antibody Yields
Industry-leading



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