TXTL Antibody/DS Kit

Cell-free expression of active antibodies and proteins with disulfide bonds



Just add DNA to express analysis-ready antibodies or target proteins in hours!



Rapidly produce active antibody constructs with myTXTL Antibody/DS Kit

Antibody constructs representing a VHH, ScFv, Fab, and full IgG were expressed from a T7 promoter for 16 hours using the myTXTL Antibody/DS Kit. Analysis by antigen pull-down and SDS-PAGE visualization was completed less than 24 hours from myTXTL reaction start time. All antibody constructs successfully bound antigen.

Figure 1. SDS-PAGE visualization of antibody: antigen pull-down from dilute myTXTL reactions. Antibodies were expressed in myTXTL Antibody/DS Master Mix and complexed with antigen prior to magnetic bead purification by His-tag (lane 1-2), Twin-Strep-tag[™] (lane 3), and Protein A (lane 4).

myTXTL Antibody/DS Kits yield industry-leading performance

myTXTL Antibody/DS Master Mix and competitor kits were used to express Trastuzumab antibody constructs (Fab/IgG), FDA approved Reteplase, and Gaussia Luciferase (GLucDura). myTXTL Antibody/DS Master Mix expressed the greatest concentration of all four proteins.

Figure 2. Comparison of protein concentrations following cell-free expression. Expression of active protein was quantified by activity assay alongside a standard for Reteplase, GLucDura, and IgG Trastuzumab. Fab concentration was determined following bead purification.



Lane 2 a. HER2 b. ScFv Trastuzumab

<u>Lane 3</u> a. HER2 b. Fab Trastuzumab

Lane 4 a. HER2 b. IgG Trastuzumab c. Heavy chain d. Light chain





"The yield is impressive, and it is so much less work than purifying proteins from liters of E. coli culture. That is definitely an advantage and will save time." Somnath Mukherjee, Research Professional, University of Chicago, Illinois



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Large scale ScFv expression and evaluation

A Trastuzumab ScFv-His construct was expressed for 16 hrs in 400 uL myTXTL Antibody/DS Master Mix and purified with Magnetic NTA beads to yield over 100 μ g ScFv. This yield was enough to perform both a multipoint ELISA and SPR Analysis (Figure 3). The K_D of 14.5 nM determined for myTXTL-produced ScFv is comparable to the FDA listed K_D of 5 nM for Trastuzumab IgG.



Figure 3. Trastuzumab ScFv and HER2 binding analysis by SPR and ELISA. SPR was conducted with immobilized ScFv and 12.5 nM - 200 nM HER2 injections. ELISA performed using HER2 coated wells. Data generated by Somnath Mukherjee, University of Chicago.

in vivo Challenges

- Expressing Ab/DS proteins
- Multiday workflow
- Lysis/purification
- Cloning

myTXTL Solutions!

- Simple workflow, fits automation
- Faster screening, hours not days
- Optional purification, no lysis
- Skip cloning, use linear DNA

Applications

- Antibody binding (ELISA/SPR)
- Screening
- De novo protein design testing
- Enzyme discovery/optimization

Specifications

Protein targets	VHH, ScFv, Fab, IgG, disulfide and non-disulfide bond proteins
Typical yield	5 μg to >100 μg
Reaction volume	Variable, 5 μL to >250 μL
Promoter	Any T7 or <i>E. coli</i> promoter system
Input DNA	Plasmid or Linear DNA (10-40 ng/μL)
Incubation conditions	Temp = 27° C, Time = 4–24 hours

Ordering information

Catalog #	Description
560300	myTXTL Antibody/DS Cell-Free Expression Kit, 300 μ L
561000	myTXTL Antibody/DS Cell-Free Expression Kit, 1000 μL
5610ML	myTXTL Antibody/DS Cell-Free Expression Kit, 10 mL
503002	T7 deGFP Control Plasmid
503003	T7 GLuc Control Plasmid
502138	P70 deGFP Control Plasmid

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Learn more at www.arborbiosci.com/cell-free-protein-synthesis

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