myTXTL® Antibody/DS Cell-Free Expression Kit

Cell-Free Protein Expression System

User Manual

Version 1.0 June 2024

Access additional manuals at arborbiosci.com/mytxtl-manuals

FOR RESEARCH USE ONLY. Not intended for diagnostic use.

© Daicel Arbor Biosciences
5840 Interface Drive, Suite 101
Ann Arbor, MI 48103 (USA)
+1 (734) 998-0751
info@arbor.daicel.com

CONTENTS

INTRODUCTION

Welcome to myTXTL!

Welcome to the world of myTXTL cell-free protein expression. myTXTL enables an accelerated DNA-to-protein workflow compared to *in vivo* protein expression systems. Time-consuming steps such as cloning, transformation, lysis, and protein purification can often be skipped when using myTXTL.

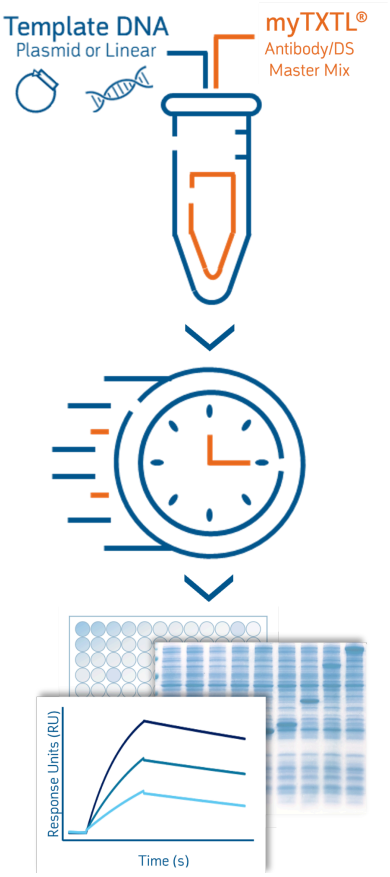
The myTXTL Antibody/DS Kit is specially formulated to **express disulfide bond-containing proteins** including VHH, ScFv, Fab and IgG constructs. myTXTL supports both plasmid and linear DNA templates, and one or several proteins can be expressed in the same reaction. Yields are typically sufficient for a variety of downstream assays like ELISA and SPR. Well-expressed disulfide bond-containing proteins can reach yields over 0.25 mg/mL in just 6 to 16 hours.

myTXTL Antibody/DS Master Mix is derived from an *E. coli* lysate and supports all constitutive or inducible promoters used in *E. coli*-based protein expression systems. Expression of proteins under regulation of a T7 promoter can be achieved by including the DS Helper Plasmid in your myTXTL reaction.

For maximum yield expression of proteins without disulfide bonds, check out our myTXTL Pro Cell-Free Expression Kit

myTXTL Antibody/DS procedure overview

1. Template DNA encoding one or more target proteins, Helper Plasmid (for T7 expression), and Master Mix are combined into myTXTL Antibody/DS reactions.
2. Reactions are incubated at 27°C for 1–16 hrs, depending on desired protein yield.
3. Resulting protein can be used and analyzed however you see fit!



Intended use

The myTXTL Antibody/DS Cell-Free Expression Kit is intended for **research use only.**
This product is not intended for disease diagnosis, prevention, or treatment.

myTXTL Antibody/DS kits, accessories, and storage

myTXTL Antibody/DS Master Mix should be stored at -80°C. All other components can be stored at ≤ -20°C.

Catalog #	Description	Contents	Quantity	Storage Temp.
560300	myTXTL Antibody/DS Cell-Free Expression Kit, 300 µL	myTXTL Antibody/DS Master Mix	3 × 100 µL	-80°C
		T7 GLuc Control Plasmid	1 × 25 µL	≤ -20°C
		DS Helper Plasmid	1 × 20 µL	≤ -20°C
561000	myTXTL Antibody/DS Cell-Free Expression Kit, 1000 µL	myTXTL Antibody/DS Master Mix	10 × 100 µL	-80°C
		T7 GLuc Control Plasmid	1 × 25 µL	≤ -20°C
		DS Helper Plasmid	3 × 20 µL	≤ -20°C
5610ML	myTXTL Antibody/DS Cell-Free Expression Kit, 10 mL	myTXTL Antibody/DS Master Mix	10 × 1 mL	-80°C
		T7 GLuc Control Plasmid	2 × 25 µL	≤ -20°C
		DS Helper Plasmid	3 × 250 µL	≤ -20°C
503003	T7 GLuc Control Plasmid	T7 GLuc Control Plasmid	1 × 25 µL	≤ -20°C

T7 GLuc Control Plasmid is supplied at 24 nM. DS Helper Plasmid is supplied at 7.2 nM.

Additional reagents required

Reagent	Notes
Nuclease-free, molecular biology-grade water	
Plasmid or linear DNA template encoding the desired target protein(s)	24 nM concentration in ≤ 50 mM Tris, ≤ 0.5 mM EDTA

Equipment required

Equipment
Sterile, nuclease-free 2 mL microcentrifuge tubes, or 0.2 mL PCR tubes/strips, or multi-well plates with tight lids
Nuclease-free filter tips and pipets capable of pipetting 0.5–100 µL
Thermal block or shaker with heated lid, or enclosed incubator with water or bead bath suitable for reaction vessels
Mini-centrifuge and/or microcentrifuge
Vortex mixer

DNA TEMPLATE PREPARATION

Compatible templates

myTXTL supports expression from both plasmid and linear DNA.

Template design

Built with an *E. coli* lysate, myTXTL supports protein expression from templates that contain elements recognized by the *E. coli* transcription and translation machinery. The kit also supports T7 promoter-based expression when the optional DS Helper Plasmid is included in the reaction mix. Therefore **expression templates must contain the following:**

1. *Linear templates only:* 50–100 bp leader sequence free of regulatory elements
2. An inducible or constitutive promoter (T7 or native *E. coli*)
3. A ribosomal binding site
4. A start codon
5. An open reading frame (ORF) for the protein of interest
6. A stop codon
7. A terminator
8. *Linear templates only:* 50–100 bp trailer sequence free of regulatory elements

DNA purity

Impurities can interfere with cell-free protein expression. Use a UV-absorbance device to quantify your template and measure absorption wavelength ratios. **DNA templates should have a 260:280 ratio of 1.8–2.2, and a 260:230 ratio of 2.0–2.3.**

When preparing your own plasmids from cells, use a dedicated plasmid DNA extraction kit like the ZymoPURE™ Plasmid Miniprep Kit (Zymo Research). In many cases, plasmids benefit from an additional round of purification using a kit like the Zymo DNA Clean and Concentrator Kit (Zymo Research). Linear templates generated with PCR or Golden Gate Assembly should also be purified with a PCR purification kit. **Elute templates in nuclease-free water or ≤ 50 mM Tris, ≤ 0.5 mM EDTA (pH 7.4–8.0).**

Template concentration

When using single templates for expression, we recommend diluting/concentrating your templates to 24 nM (about 50 ng/µL for a 3.5 Kbp molecule). When expressing using two templates simultaneously (e.g, heavy and light chains from separate DNA templates to make IgG), we recommend a final 48 nM concentration of each template (see *Appendix D*). If you concentrate your template to reach these specifications, ensure that the **final concentration of Tris is ≤ 50 mM, and EDTA ≤ 0.5 mM.**

Optimal or minimum template concentration for your application may vary.

BEST PRACTICES

DNA purity and concentration

For best performance, follow the DNA purification process and template concentration guidelines outlined in *DNA TEMPLATE PREPARATION*.

Positive control reaction

The T7 GLuc Control Plasmid can be used as template in a positive control reaction. If desired, it can be used to generate a linear DNA template by PCR with primers listed in *Appendix C*. Incubation of the control reaction for 16 h at 27°C should produce sufficient *Gaussia* luciferase enzyme to visualize by SDS-PAGE without purification. GLuc enzymatic activity can also be evaluated by a luminescence assay. See *Appendix A* for further instructions on evaluating control reactions.

Incubation platform

Use a reaction incubation system that prevents condensation on tube walls for the extent of your planned incubation time at 27°C. Thermo-shakers with heated lids are the most flexible, but several setups will work well. If using a water bath, use the cover for the duration of the incubation.

Reaction vessel and agitation

Choice of vessel and agitation depends on the chosen reaction volume:

Reaction volume	Recommended vessel	Agitation
< 6 µL	0.2 mL tubes or plates, or 384-well plates	None
6–25 µL	2 mL tube, 96-well plate	None
26–100 µL	2 mL tube, 24-well plate	650 rpm
> 100 µL	6–24-well plate	300 rpm

Bubbles

Avoid bubbles by pipetting slowly and minimize vortexing myTXTL Master Mix and reactions.

Pilot testing

Expression efficiency varies among proteins. We recommend testing the expression of your proteins in pilot experiments to determine the reaction volume and incubation time suitable to your project goals.

Full-length antibodies

A typical procedure for expressing IgG heavy and light chains in myTXTL uses a template molar ratio of 1:1. See *Appendix D* for an example setup for expressing full-length IgG.

His-tag purification

Avoid use of imidazole-containing binding and wash buffers when purifying His-tagged proteins expressed in myTXTL reactions.

REACTION PROCEDURE

The following describes a general-purpose myTXTL Antibody/DS reaction setup. Review and follow the *BEST PRACTICES* and guidelines in *DNA TEMPLATE PREPARATION*. Optimal conditions for your application may require experimentation to identify. Please refer to our FAQs or reach out to our technical support team (techsupport@arbor.daicel.com) with any questions.

1. **Pick a reaction volume and reaction vessel.** Reactions are typically set up between 6 µL and 100 µL final volume. For the first attempt expressing a target protein, start with 12 µL reactions. See *BEST PRACTICES - Reaction vessel and agitation* for guidelines.

2. **Preheat the incubator, bath, or thermomixer to 27°C.** Use a heated lid if it is an option, set similar to reaction temperature. If using a bath, ensure the reaction vessel is fully surrounded by water or beads to minimize condensation over the course of incubation. Water baths should be covered.

3. **Thaw all kit components and templates at room temperature, then immediately transfer to ice.**

4. **Immediately before use, briefly (~1 s) spin down the myTXTL Antibody/DS Master Mix with a mini-centrifuge. Then, mix well by pipetting with pipette set to about 50% of the Master Mix volume to ensure homogeneity and avoid bubbles.**

5. **Assemble the myTXTL reaction(s).** Combine the components below in the order indicated. Component volumes are indicated for two example reaction sizes, while a reaction of size V µL can be built by multiplying V by the indicated ratios per component.

Component	12 µL reaction	50 µL reaction	V µL reaction
Antibody/DS Master Mix	9.0 µL	37.5 µL	0.75 × V µL
DS Helper Plasmid*	0.5 µL	2.0 µL	0.04 × V µL
Template DNA at 24 nM†	2.5 µL	10.5 µL	0.21 × V µL
TOTAL VOLUME	**12.0 µL**	**50.0 µL**	**V µL**

** DS Helper Plasmid is necessary when using a T7 promoter, such as the T7 GLuc Control Plasmid. If you are using a non-T7 promoter system, DS Helper Plasmid can be replaced with nuclease-free water.*

† We recommend a 24 nM stock concentration and a final 5 nM target concentration of each template in the myTXTL reaction. See Appendix D for guidelines when expressing from two templates simultaneously at a molar ratio of 1:1. For templates of higher starting concentration, make up for remaining volume with nuclease-free water. Optimal template concentration for your application may vary. ***For a negative control reaction, replace template DNA volume with nuclease-free water.***

6. **Briefly vortex and mini-centrifuge (~1 s each step) the assembled myTXTL reaction.**

REACTION PROCEDURE (CONTINUED)

7. **Incubate the myTXTL reaction(s) at 27°C for up to 16 hours. Agitate the reactions during incubation if larger than 25 µL.** See *BEST PRACTICES - Reaction vessel and agitation* for guidelines.

8. **Stop the myTXTL reaction(s) by placing the reactions on ice.**

9. **Evaluate the positive control reaction(s).** If the T7 GLuc Control Plasmid was used, expression of GLuc (21.3 kDa) can be visualized by SDS-PAGE. See *Appendix A* for further instructions on evaluating control reactions.

10. **Evaluate the quantity, affinity, or activity of your target protein.** Depending on your downstream assay, purification may not be necessary. See *Appendix A* and *Appendix B* for guidance on visualizing myTXTL-expressed proteins by SDS-PAGE.

    If you plan to purify your protein by His-tag affinity pulldown, avoid binding and wash buffers that contain imidazole.

APPENDICES

Appendix A. Evaluating GLuc control reactions

The myTXTL Antibody/DS Cell-Free Expression Kits include the T7 GLuc Control Plasmid. It encodes for a *Gaussia* luciferase Dura protein that bears an 8XHis-tag at the N-terminus and 5 disulfide bonds. The control plasmid can be used in either its source or linear form as a positive control template to help evaluate the performance of the myTXTL reactions.

Analysis of GLuc by SDS-PAGE

1. After executing a GLuc Control myTXTL reaction, conduct the protocol in *Appendix B*, preparing duplicate 1.5 µL samples of the supernatant from the GLuc myTXTL reaction. At step 7, resuspend one sample pellet in 18 µL of 1X **non-reducing** SDS-PAGE loading dye and the other sample in 18 µL of 1X **reducing** SDS-PAGE loading dye. Proceed to boiling and loading these samples as described.
2. Non-reduced and reduced samples should migrate at different rates. The non-reduced sample should run faster and appear smaller than the reduced sample, which should run close to 21.3 kDa.

GLuc activity assay

Centrifuge the myTXTL control reaction for 3 minutes at 16,000 × *g* or greater. Combine 2 µL of the centrifuged supernatant with 198 µL PBS (pH 7.4) and mix thoroughly. Then use a system such as the NanoLight™ GLuc GLOW Assay (NanoLight Technology, Cat. no. 320) to measure the concentration of *Gaussia* luciferase by mixing 10 µL of the 100-fold diluted myTXTL sample with 50 µL of the NanoFuel GLOW working solution. Measure the luminescence with a luminometer after 5 minutes. If the control reaction was incubated for at least 16 h at 27°C using the recommended control template concentration, the final luciferase concentration of the original non-dilute reaction should be close to 10 µM or 0.2 mg/mL.

Appendix B. Preparing myTXTL Antibody/DS reactions for SDS-PAGE

Disulfide bond formation can be checked by comparison of non-reduced and reduced samples by SDS-PAGE. The target protein band should shift in apparent mass depending on whether disulfide bonds are intact or reduced. Note that for proteins such as IgG and Fab, their component polypeptide chains will dissociate in reduced samples and appear as distinct bands, while non-reduced samples will remain intact.

Disulfide bond-containing proteins often require purification prior to SDS-PAGE in order for the target protein to be visually discernible from proteins native to the myTXTL Antibody/DS Master Mix. After purification, load the equivalent of 2–6 µL of the original myTXTL reaction volume for SDS-PAGE analysis.

Exceptionally well-expressed proteins may not require purification before SDS-PAGE. However, proteins in this category that are smaller than 25 kDa may still benefit from acetone precipitation prior to loading. The following protocol describes acetone precipitation and SDS-PAGE analysis of the equivalent of 1 µL of myTXTL reaction:

1. Centrifuge the purified or non-purified myTXTL reaction(s) for 3 minutes at 16,000 × *g* or greater.
2. Transfer 1.5 µL of the myTXTL reaction supernatant into a 1.5 mL microcentrifuge tube.
3. Add 15 µL of ice-cold acetone to this 1.5 µL of myTXTL reaction supernatant to precipitate proteins.
4. Centrifuge the precipitated reactions for 3 minutes at 16,000 × *g* or greater.
5. Remove the supernatant from the microcentrifuge tube(s) and pipette on a paper towel to discard.
6. Allow the protein pellet(s) to air dry with the lid of the microcentrifuge tube(s) open for 10 minutes at room temperature.
7. Resuspend the pellet(s) in 18 µL of 1X **non-reducing** SDS-PAGE loading dye and boil at 95°C for 3–5 minutes.
8. Briefly cool the sample(s) on ice.
9. Briefly centrifuge the SDS-PAGE sample(s) to collect the contents.
10. Load 12 µL of the SDS-PAGE sample(s) into a SDS-polyacrylamide gel to visualize the equivalent of 1 µL of the endpoint myTXTL reaction.

Appendix C. Converting control plasmid to linear template

When using linear DNA templates for protein expression, a linear positive control can be helpful to evaluate the behavior of the system. Linear control template can be generated using the T7 GLuc Control Plasmid as template (final product of 969 bp) in a PCR amplification with the following primers:

T7LPfor: CGGCCACGATGCGTCC **T7LPrev**: CGACCGCTTTGGCCG

These amplify the entire promoter-terminator span while retaining both leader and trailer sequence. See *DNA TEMPLATE PREPARATION - DNA purity* for guidance on purifying the amplicon.

To assist in DNA design, DNA plasmid sequences and examples of linear DNA templates can be found on our website (www.arborbiosci.com/mytxtl-sequences).

Appendix D. Expression of IgG

The myTXTL Antibody/DS kit can be used to express IgG from either plasmid or linear DNA templates. Below is a sample reaction setup for conducting IgG expression from two separate DNA templates, one for the heavy chain (HC) and one for the light chain (LC) at a 1:1 molar ratio HC:LC in the myTXTL reaction. This table would replace the table in *Reaction Procedure*, otherwise follow the steps of that section.

Component	12 µL reaction	50 µL reaction	*V* µL reaction
Antibody/DS Master Mix	9.0 µL	37.5 µL	0.75 × *V* µL
DS Helper Plasmid*	0.5 µL	2.0 µL	0.04 × *V* µL
IgG HC DNA at 48 nM †	1.25 µL	5.25 µL	0.11 × *V* µL
IgG LC DNA at 48 nM †	1.25 µL	5.25 µL	0.11 × *V* µL
TOTAL VOLUME	12.0 µL	50.0 µL	*V* µL

** DS Helper Plasmid is necessary when using a T7 promoter, such as the T7 GLuc Control Plasmid. If you are using a non-T7 promoter system, DS Helper Plasmid can be replaced with nuclease-free water.*

† We recommend a 48 nM stock concentration for a final 5 nM concentration of each HC and LC DNA in the myTXTL reaction. For templates of higher starting concentration, make up for remaining volume with nuclease-free water. Optimal template concentration for your application may vary. For a negative control reaction, replace template DNA volume with nuclease-free water.

TROUBLESHOOTING

Protein expression efficiency in any system can vary between constructs and can be sensitive to a number of variables. Below is a non-exhaustive list of potential problematic observations, common causes thereof, and associated potential solutions.

1. **My protein fails to express, but the positive control expresses well.**
   Some common causes and potential solutions of poor or failed expression are:
   a. The template DNA is not of sufficient purity: This is the most common cause of a poor performing myTXTL reaction. Please carefully follow the guidelines in *DNA TEMPLATE PREPARATION - DNA purity*. To check the performance of an in-house plasmid purification protocol, the myTXTL T7 GLuc Control Plasmid can be propagated in *E. coli* (Amp^r) and purified. Compare GLuc expression from your in-house generated plasmid to the myTXTL T7 GLuc Control Plasmid expression on an SDS-PAGE, loading equivalent myTXTL reaction volume. If you get similar expression results, your in-house purification protocol is likely okay.
   b. The template is lacking critical structural elements: See *DNA TEMPLATE PREPARATION - Template design*.
   c. The protein is insoluble: Following expression, check if the protein is insoluble:
      i. Centrifuge myTXTL reaction(s) 3 min at 16,000 × *g* or greater, then transfer the full supernatant to a new vessel.
      ii. Using a volume of 1X reducing SDS-PAGE loading dye equal to the reaction supernatant, resuspend insoluble material by pipetting repeatedly over the reaction vessel surfaces that had been in contact with myTXTL reaction. Ensure no insoluble material is visible.
      iii. Dilute 1 or 2 µL of resuspended sample into 11 or 10 µL 1X reducing SDS-PAGE loading dye and boil at 95°C for 5 minutes.
      iv. Allow the sample to cool, then load 12 µL into an SDS-PAGE gel to visualize 1–2 µL reaction equivalent of the insoluble material.
   d. The protein requires post-translational modification: The myTXTL system does not enable post-translational modifications.
   e. The promoter used is not an endogenous *E. coli* promoter or T7: Switch promoter systems to T7 or one commonly used in *E. coli*.
   f. The protein expression is under the control of an inducer: Add the inducer to the reaction, explore increased plasmid concentrations up to 20 nM if adding the inducer does not produce desired yields. See table below:

Promoter	Inducer	Recommended inducer concentration in final reaction	Recommended plasmid template concentration in final reaction
T7lac	IPTG	1 mM	10 nM
TetA	aTc	20 µg/mL	20 nM
araBAD	L-Arabinose	2%	20 nM

g. One or more reagents, potentially introduced with the template, compromised the reaction: The following shows the maximum tolerable concentrations of various additives in the final myTXTL reaction:

Glycerol:	0.1%
DMSO:	1.0%
EDTA:	0.1 mM
Tris-HCl (pH 8):	50 mM
$CaCl_2$:	1.0 mM
$MgCl_2$:	1.0 mM
NaCl:	50 mM

2. The positive control failed to express.

a. The reactions were over- or under-agitated: Ensure that *BEST PRACTICES - Reaction vessel and agitation* was closely followed.

b. Kit components were not stored at the proper temperatures: myTXTL Antibody/DS Master Mix must be stored at -80°C, while all other components can be stored at -20°C or lower.

3. I can see my protein by SDS-PAGE, but it does not demonstrate the expected level of activity.

a. The protein requires post-translational modifications for activity: The myTXTL system does not enable post-translational modifications.

b. The protein requires different reaction conditions: Lower the incubation temperature, include co-factors or additional chaperones to promote proper folding.

4. After His-tag purification, I have low amounts or no detectable protein.

a. The binding and/or wash buffers contained imidazole: Check the formulation of the buffers used and replace them with imidazole-free versions.