myTXTL® Pro Cell-Free Expression Kit

Cell-Free Protein Expression System

User Manual

Version 1.0 June 2024

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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 Pro Kit uses linear or plasmid DNA templates to **express proteins that lack disulfide bonds**. It can express one or several proteins in the same reaction, and can produce enough protein for a variety of downstream assay types. Well-expressed proteins can reach yields over 1 mg/mL in just 6 to 16 hours.

myTXTL Pro 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 Pro Helper Plasmid in your myTXTL reaction.

For expression of proteins containing disulfide bonds, check out our myTXTL Antibody/DS Cell-Free Expression Kit

myTXTL Pro procedure overview

1. Template DNA encoding one or more target proteins, Helper Plasmid (for T7 expression), and Master Mix are combined into myTXTL Pro 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 Pro Cell-Free Expression Kit is intended for **research use only**.
This product is not intended for disease diagnosis, prevention, or treatment.

myTXTL Pro kits, accessories, and storage

myTXTL Pro Master Mix must be stored at -80°C, while all other components can be stored at ≤ -20°C.

Catalog #	Description	Contents	Quantity	Storage Temp.
540300	myTXTL Pro Cell-Free Expression Kit, 300 µL	myTXTL Pro Master Mix	3 × 100 µL	-80°C
		T7 deGFP Control Plasmid	1 × 25 µL	≤ -20°C
		Pro Helper Plasmid	1 × 20 µL	≤ -20°C
541000	myTXTL Pro Cell-Free Expression Kit, 1000 µL	myTXTL Pro Master Mix	10 × 100 µL	-80°C
		T7 deGFP Control Plasmid	1 × 25 µL	≤ -20°C
		Pro Helper Plasmid	3 × 20 µL	≤ -20°C
5410ML	myTXTL Pro Cell-Free Expression Kit, 10 mL	myTXTL Pro Master Mix	10 × 1 mL	-80°C
		T7 deGFP Control Plasmid	2 × 25 µL	≤ -20°C
		Pro Helper Plasmid	3 × 250 µL	≤ -20°C
503002	T7 deGFP Control Plasmid	T7 deGFP Control Plasmid	1 × 25 µL	≤ -20°C
502138	P70 deGFP Control Plasmid	P70 deGFP Control Plasmid	1 × 30 µL	≤ -20°C

T7 deGFP Control Plasmid is supplied at 24 nM. Pro Helper Plasmid is supplied at 2.4 nM. P70 deGFP Control Plasmid is supplied at 20 nM.

Additional reagents required

Reagent	Notes
Nuclease-free, molecular biology-grade water	
Plasmid or linear DNA template encoding the desired target protein(s)	plasmid DNA: 24 nM; linear DNA: 96 nM

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 Pro 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 10 mM Tris, ≤ 0.1 mM EDTA (pH 7.4–8.0).**

Template concentration

When using plasmid templates, we recommend diluting/concentrating your templates to 24 nM (about 50 ng/µL for a 3.5 Kbp molecule). For linear templates, use 96 nM (about 200 ng/µL for 3.5 Kbp). 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 deGFP Control Plasmid can be used as a template for a positive control reaction. For expression using a native *E. coli* promoter system, the P70 deGFP Control Plasmid (Cat. No. 502138) can be used instead. If desired, the T7 deGFP Control plasmid can be used to generate linear DNA templates by PCR with primers listed in *Appendix C*. Successful expression will turn the reaction visibly fluorescent green. When positive control reactions are incubated at 27°C for at least 4 hours, the deGFP protein (25.4 kDa) should be visible with SDS-PAGE without purification. After 16 hours, you should expect a final concentration of about 1 mg/mL deGFP. 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

These are dependent on the user's chosen reaction volume and are designed to maintain sufficient oxygenation of the system.

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

Pipette slowly and minimize vortexing myTXTL Pro Master Mix and myTXTL reactions, as bubbles can significantly reduce expression efficiency.

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.

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 Pro 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 the 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 Pro Master Mix with a mini-centrifuge. Then, mix well by pipetting with pipette set to about 50% 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
Pro Master Mix	9.0 µL	37.5 µL	0.75 × V µL
Pro Helper Plasmid*	0.5 µL	2.0 µL	0.04 × V µL
Template DNA at 24 or 96 nM †	2.5 µL	10.5 µL	0.21 × V µL
TOTAL VOLUME	**12.0 µL**	**50.0 µL**	**V µL**

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

† For plasmid DNA templates, we recommend a 24 nM stock concentration for a final 5 nM concentration in the myTXTL reaction. For linear DNA templates, we recommend a 96 nM stock concentration for a final 20 nM concentration 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.***

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 you included a positive control reaction using T7 deGFP Control Plasmid, P70 deGFP Control Plasmid, or a linear derivative thereof, successful expression will turn the reaction visibly fluorescent green. When positive control reactions are incubated at 27°C for at least 4 hours, the deGFP protein (25.4 kDa) should be visible on an SDS-PAGE gel without purification. After 16 hours, you should expect about 1 mg/mL deGFP. 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 B* for guidance on visualizing proteins smaller than 25 kDa by SDS-PAGE.

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

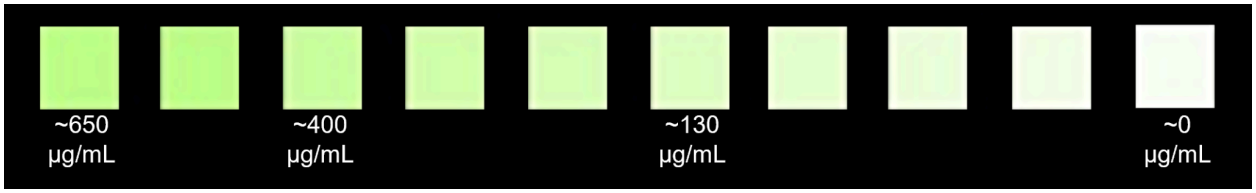
APPENDICES

Appendix A. Evaluating deGFP control reactions

The myTXTL Pro Cell-Free Expression Kits include the T7 deGFP Control Plasmid, which encodes an engineered version of enhanced green fluorescent protein (deGFP). The control plasmid can be used in either its source or linear forms as a positive control template to help evaluate the performance of the myTXTL reactions. The deGFP can provide both a qualitative (visual) indicator of successful expression, and a quantitative measure of expression efficiency.

Visual analysis of deGFP positive control reaction

Centrifuge the control reaction at room temperature for 3 minutes at 16,000 × *g* or greater. Lay the tube on a white piece of paper. Compare the reaction's green color intensity to the color strip below to approximate the deGFP concentration. If the reactions were incubated for at least 16 h at 27°C using the recommended control template concentration, the color should be more intense than the leftmost swatch.



Quantitative analysis of deGFP positive control reaction

The following method allows you to quantify the expression efficiency of the control reaction using a spectrophotometer.

1. Prepare dilutions of the T7 deGFP Positive Control reaction
    a. Centrifuge the control reaction(s) for 3 minutes at 16,000 × *g* or greater.
    b. Prepare triplicate 10-fold dilutions of the control reaction using PBS as a diluent.
2. Measure absorbance at 488 nm wavelength using a UV spectrophotometer
    a. Blank the device using PBS
    b. Measure the positive control reaction dilutions and calculate an average absorbance. *A*.
3. Calculate the average concentration of deGFP in the positive control reaction
    a. Multiply *A* by 4.616 to estimate mg/mL deGFP in the source (pre-dilution) control reactions (assumes a path length of 1 cm; check the path length of your spectrophotometer).
    b. If the reactions were incubated for at least 16 h at 27°C using the recommended control template concentration, the deGFP concentration should be close to 1 mg/mL.

Appendix B. Preparing myTXTL Pro reactions for SDS-PAGE

Proteins below 25 kDa require additional processing in order to be properly visualized by SDS-PAGE. We recommend that the following acetone precipitation protocol is performed for any such proteins. The following protocol supports the visualization of 1 µL of the endpoint myTXTL reaction.

1. Centrifuge the 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 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 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 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 deGFP Control Plasmid as template (final product of 1.05 kbp) 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).

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 deGFP Control Plasmid can be propagated in *E. coli* (Amp^r) and purified. Compare deGFP expression from your in-house generated plasmid to the myTXTL T7 deGFP Control Plasmid reaction to evaluate your purification protocol.
   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 contains disulfide bonds: Use the myTXTL Antibody/DS Cell-Free Expression Kit (Cat. Nos: 560300, 561000, 5610ML), which is designed for disulfide bond-containing proteins.
   e. The protein requires post-translational modification: The myTXTL system does not enable post-translational modifications.
   f. The promoter used is not an endogenous *E. coli* promoter or T7: Switch promoter systems to T7 or one commonly used in *E. coli*.
   g. 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

h. 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 reach ~1 mg/mL concentration.

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 Pro 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 in SDS-PAGE, but it does not demonstrate the expected level of activity.

a. The protein contains disulfide bonds: Use the myTXTL Antibody/DS Cell-Free Expression Kit. (Cat. Nos: 560300, 561000, 5610ML), which is designed for disulfide bond-containing proteins.

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

c. 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.