5692 Plymouth Road
Ann Arbor, MI 48105 (USA)
+1 (734) 998-0751
info@mycroarray.com

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MYtxtl®

CELL-FREE TRANSCRIPTION-TRANSLATION KIT

MYtxtl™ is an easy-to-use kit for rapid cell-free protein expression and the execution of complex multi-gene molecular programs. *In vitro* transcription-translation (TX-TL) is initiated by simply mixing our all-in-one MYtxtl™ Master Mix containing the entire TX-TL machinery with a DNA template in a single tube bypassing time-consuming molecular biology procedures such as bacterial transformation and clone selection. MYtxtl™ provides you with your protein of interest in the microgram to milligram per mL range within only a few hours, after which the complete TX-TL reaction can further processed directly in downstream applications. These can include either an immediate use in an activity assay or protein purification e.g. via affinity chromatography, making MYtxtl™ ideal for high-throughput technologies.

Overview

MYtxtl™ is based on an *in vitro* transcription-translation platform developed by Prof. Vincent Noireaux at the University of Minnesota (USA) that entirely relies on the endogenous TX-TL machinery of *E. coli* which employs the core RNA polymerase and sigma factor 70 (σ^{70}) as basic transcription factors. MYtxtl™ comes as an all-in-one Master Mix containing all TX-TL reagents (*E. coli* cell extract, amino acids, and energy buffer) and — for the expression of a single protein — only requires the addition of a DNA template comprising the gene of interest under transcriptional control of a σ^{70} specific promoter (P_{70}).

In addition, gene expression under the control of the bacteriophage T7 promoter/operator system is also feasible by using a two-plasmid system: one for the expression of T7 RNA polymerase under transcriptional control of promoter P_{70}, the other comprising the gene of interest downstream of the T7 promoter. For setting up multi-stage activation cascades (gene circuitries), MYtxtl™ can be complemented with a plethora of stable and degradable exogenous transcription factors available in our Toolbox 2.0 plasmid collection (for more information please go to www.mycroarray.com).

Currently, our Sigma 70 Master Mix Kit is available in two different sizes:

Product	Catalog number	Contents
Sigma 70 Master Mix Kit	MYtxtl-70-24-M	Contains **3** tubes (75 µL) of Sigma 70 Master Mix (cell extract, amino acids, energy buffer) to set up 24 TX-TL reactions (each 12 µL)
	MYtxtl-70-96-M	Contains **12** tubes (75 µL) of Sigma 70 Master Mix (cell extract, amino acids, energy buffer) to set up 96 TX-TL reactions (each 12 µL)

Kit components

- MYtxtl™ Sigma 70 Master Mix (75 µL each tube)
- P70a-eGFP control plasmid (10 µL, 20 nM)

Equipment (not included in MYtxtl™ kit)

- Nuclease-free, barrier tips and pipets capable of pipetting 0 – 100 µL
- Sterile and nuclease-free 1.5 or 2.0-mL tubes
- Incubator, thermoblock or water bath
- Table-top microcentrifuge
- Vortex mixer
- Fluorescence plate reader (e.g. Tecan Genios; only needed for eGFP quantification)
- Black, optical-bottom 384-well plate (e.g. Nunc; only needed for eGFP quantification)

Reagents (not included in MYtxtl™ kit)

- Nuclease-free water
- Phosphate-buffered saline (1x PBS; only needed for eGFP quantification)
- Recombinant eGFP (Cell Biolab, # STA-201; only needed for eGFP quantification)

Storage and Stability Information

The MYtxtl™ kit, containing the MYtxtl™ Sigma 70 Master Mix and the P70a-eGFP control plasmid, is shipped on dry ice. Upon receipt, the MYtxtl™ Sigma 70 Master Mix must immediately be stored at –80 °C. Since the P70a-eGFP control plasmid is suspended in nuclease-free water, we recommend a storage temperature of –20 °C to prevent its degradation. Once thawed, the MYtxtl™ Sigma 70 Master Mix should be stored on ice and used within 4 hours. One thaw-and-freeze cycle is possible for the MYtxtl™ Sigma 70 Master Mix without loss of protein production efficiency. When stored and handled under these conditions, the MYtxtl™ kit is stable for 6 months upon arrival.

Recommendations for Preparation of Template DNA

In vitro protein production is highly sensitive to DNA purity. For reliable and reproducible results, template DNA should be free of nucleases (DNases, RNases) and inhibitors of the TX-TL machinery (EDTA, ethidium bromide, SDS, Cl^- ions).

Preparation of plasmid DNA with standard commercial kits usually involves sample treatment with RNase A which may not be completely removed during downstream processing. Thus, we strongly recommend subjecting the prepared DNA to standard phenol-chloroform purification and ethanol precipitation. Ideally, template DNA is suspended in nuclease-free water and should be free of potential inhibitors such as NaCl, glycerol, EDTA and magnesium or potassium salt.

The final concentration for the provided P70a-eGFP control plasmid in a TX-TL reaction is 5 nM (60 fmol). Although optimum concentration of your template DNA may vary depending on the promoter strength and your protein of interest, we recommend 5 nM DNA template as a starting concentration for your TX-TL reaction.

For convenience, the control plasmid P70a-eGFP can also be used as a cloning vector for a target gene (Figure 1). It contains the eGFP gene under the transcriptional control of the lambda phage promoter Pr (P_{70a}), the *E. coli* terminator T500 and the ampicillin resistance gene. The eGFP gene is flanked by a *Nco*I and *Xho*I restriction sites, respectively which can be used to replace it in-frame with any gene sequence of your choice following an easy cut-and-paste procedure. The complete plasmid sequence is available upon request. We highly recommend conducting amplification of P70a-eGFP plasmid (and its derivatives) in *E.coli* KL740, since it — when grown below 30 °C — over-expresses the temperature sensitive Lambda repressor Cl857 necessary for an efficient repression of gene synthesis and high plasmid stability.

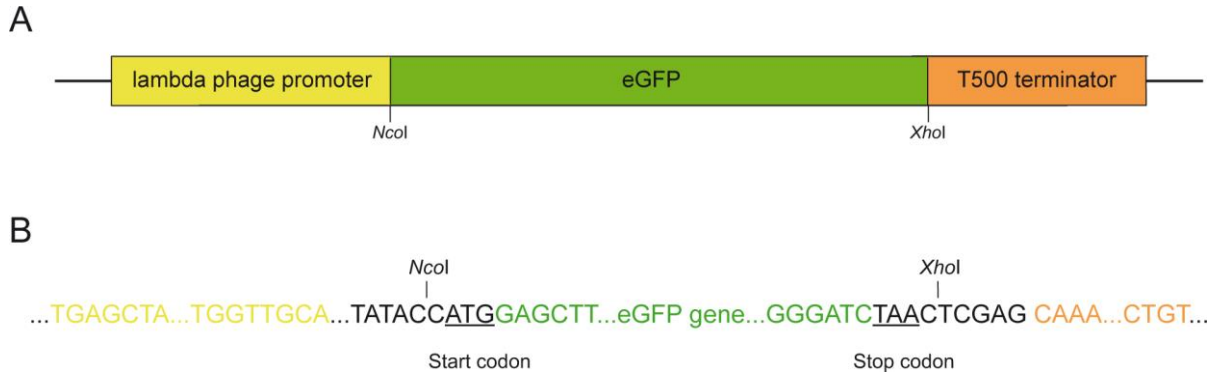

Figure 1. Schematic drawing of P70a-eGFP control plasmid (A) also showing an excerpt of the nucleotide sequence in proximity to important cloning sites (B).

Procedure

Before starting

- Always wear gloves and use nuclease-free reaction tubes as well as barrier tips when working with the MYtxtl™ kit, since it is extremely sensitive to nuclease contamination.
- Completely thaw all kit components on ice before use and keep them on ice while setting up the MYtxtl™ reaction.
- Each tube MYtxtl™ Sigma 70 Master Mix (75 µL) is enough to prepare eight 12 µL MYtxtl™ reactions at once.
- A typical MYtxtl™ reaction should be 12 µl. Please consult with techsupport@mycroarray.com if larger volumes are needed.
- The TX-TL reaction is very sensitive to varying DNA concentration. Make sure to transfer the entire volume of the DNA template to the MYtxtl™ Sigma 70 Master Mix.
- To determine the background protein level, prepare a MYtxtl™ reaction without any template DNA (use equivalent volume of nuclease-free water instead).
- For multiple TX-TL reactions, prepare a master mix and transfer 12 µL aliquots to individual reaction tubes.
- During incubation of the TX-TL reaction, avoid any condensation on the tube lid since changes in the reaction volume could have a considerably negative effect on the kit performance.

Protein Synthesis

1) **Preheat incubator (or thermoblock or water bath) to 29 °C.**
2) **Completely thaw and store MYtxtl™ Sigma 70 Master Mix, your template DNA or/and P70a-eGFP control plasmid on ice.**
3) **Before use, gently vortex and briefly centrifuge the MYtxtl™ Sigma 70 Master Mix.**
   If any precipitate is visible, gently pipet mixture up and down to ensure homogeneity. Avoid foam formation.
4) **A) Set up a single MYtxtl™ reaction. Pipet each component in the order as depicted in Table 1 (column 1) to the bottom of a nuclease-free reaction tube**.
   Keep it on ice. To prepare a MYtxtl™ positive control reaction (Table 1, column 2), use the provided P70a-eGFP control plasmid at a final concentration of 5 nM instead of your template DNA.

B) Set up multiple MYtxtl™ reactions (Table 1, column 3).
Simply scale up from volumes needed for a single MYtxtl reaction and add at least 4 % of the total volume to compensate for pipetting errors, e.g. for eight TX-TL reactions (8x 12 = 96 µL) prepare a total volume of 100 µL.

Table 1. Pipetting scheme for MYtxtl™ reactions using plasmid DNA as template.

	Single MYtxtl™ reaction	**Single MYtxtl™ eGFP control. reaction**	**e.g. Eight MYtxtl™ reactions**
Sigma 70 Master Mix	9 µL	9 µL	75 µL
Template DNA	X µL (final: 5 nM)	–	X µL (final: 5 nM)
P70a-eGFP ctr. plasmid (20 nM)	–	3 µL (final: 5 nM)	–
Nuclease-free water	X µL	–	X µL
Total	12 µL	12 µL	100 µL

5) **Gently vortex and briefly centrifuge each MYtxtl™ reaction to collect the entire volume at the bottom of the tube.**

6) **In the case of a multiple MYtxtl™ reactions:** Gently pipet mixture up and down to ensure homogeneity, then split into 12 µL per reaction tube. Add a final centrifugation step to spin reaction down.

7) **Incubate the MYtxtl™ reaction tube(s) at 29 °C for up to 16 h.**

8) **Stop the MYtxtl™ reaction by placing the tube(s) on ice**.

9) **Subsequently analyze or process your MYtxtl™ reaction or freeze it for later use.**
MYtxtl™ reactions are directly amenable for sample analysis such as SDS-PAGE without precipitating the sample with TCA, ethanol or acetone. Since your protein of interest already exists free in solution, immediate protein purification via affinity chromatography or extraction of DNA/RNA is possible.

Evaluation of MYtxtl™ Protein Expression

For convenience, the MYtxtl™ kit is provided with the positive control plasmid P70a-eGFP encoding the enhanced green fluorescent protein (eGFP) for an easy and direct visualization of protein synthesis (Figure 2). It helps you — especially if unfamiliar with the *in vitro* TX-TL technique — to optimize the work-flow and reaction conditions.

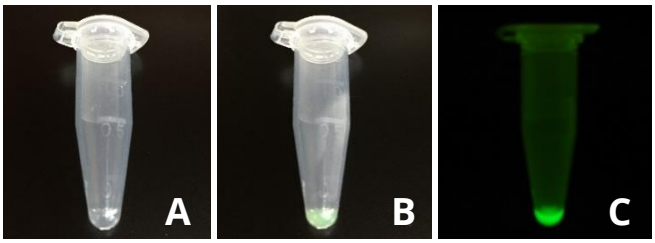

Figure 2. MYtxtl™ reaction containing the P70a-eGFP control plasmid before (A) and after (B) the incubation at 29 °C. (C) eGFP fluorescence in the MYtxtl™ reaction under UV light.

To evaluate the protein production efficiency of the MYtxtl™ kit in your lab, you can choose between two methods. After carrying out a single MYtxtl™ positive control reaction with the provided P70a-eGFP (see **Protein Synthesis**), you can decide whether to perform a **(A)** qualitative estimation of your final eGFP concentration or **(B)** quantitative analysis of your sample.

For both evaluation methods, the MYtxtl™ control reaction is centrifuged at > 16,300 g (or full speed) for 3 min (RT) using a microcentrifuge after the incubation. Then, directly proceed with sample analysis.

(A) Qualitative analysis

Compare the intensity of (green) color in your MYtxtl™ control reaction to the following standard eGFP color strip (Figure 3) to estimate the eGFP produced in your tube.

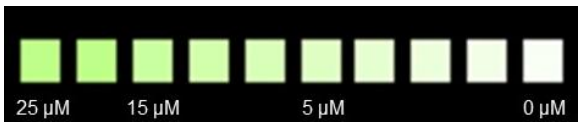

Figure 3. Color strip for the qualitative analysis of eGFP production.

(B) Quantitative analysis

1) Prepare an eGFP standard curve (0-5 µM).

- Thaw and keep the recombinant eGFP standard (Cell Biolabs, # STA-201) on ice. Determine the molar concentration of your protein solution.
- Prepare an eGFP stock solution of 5 µM in PBS (V = 70 µL) in a 1.5 mL reaction tube.
  Example: If your eGFP standard has a concentration of 30 µM, transfer 11.7 µL of the 30 µM eGFP protein solution to 58.3 µL PBS, mix thoroughly and collect mixture on the tube bottom by a short centrifugation.

- Prepare a 2-fold dilution series of eGFP in the concentration range of 0-5 µM in 1.5 mL reaction tubes (5, 2.5, 1.25, 0.63 and 0.31 µM).
  Example: To prepare a 2.5 µM eGFP solution, transfer 35 µL of the 5 µM eGFP solution to 35 µL PBS and mix thoroughly. Then take 35 µL of the 2.5 µM eGFP solution to prepare the next dilution step. Proceed to 0.31 µM eGFP (five dilution steps).
- For each dilution, transfer 10 µL/well in triplicate into a black, optical-bottom 384-well plate. Also include a Blank measurement in triplicate using PBS only.

2) Dilute the MYtxtl™ control reaction.

- Prepare a 10-fold dilution of the centrifuged MYtxtl™ control reaction in PBS. In a 1.5 mL reaction tube, add 4 µL MYtxtl™ control reaction to 36 µL PBS, mix thoroughly and collect mixture on the tube bottom by a short centrifugation.
- Transfer 10 µL/well of this diluted sample in triplicate to the same 384-well plate as the eGFP standard dilution series (see above).

3) Perform fluorescence measurement using a plate reader.

- Before the fluorescence measurement, carefully tap or briefly spin down the 384-well plate to remove any air bubbles and to equally distribute each sample in the well.
- Fluorescence reader setting: Choose an excitation and emission wavelength appropriate for eGFP measurement (e.g. emission: 488 nm, excitation: 535 nm).

4) Calculate the eGFP concentration using a calibration curve (linear regression).

- Subtract the fluorescence values of the Blank (PBS only) from that of each standard protein and MYtxtl™ control reaction.
- Plot the Blank subtracted fluorescence values of the eGFP standard (Y-axis) against their respective protein concentration (X-axis) and fit the curve to the linear regression formula (y = m*x) to determine the eGFP concentration in the MYtxtl™ control reaction (Figure 4).
- Ideally, the MYtxtl™ control reaction yields between 25 and 35 µM eGFP.

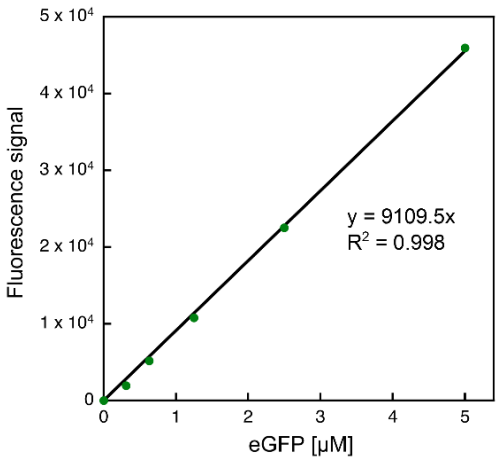

Figure 4. Example of an eGFP calibration curve to evaluate the protein production efficiency of the MYtxtl™ kit.

Frequently Ask Questions (FAQ)

1) How do I set up a negative control reaction?
   A negative control for a MYtxtl™ reaction is simply set up by replacing the template DNA by nuclease-free water.

2) Should I prepare a MYtxtl™ Master Mix if multiple reactions with the same template DNA are desired?
   Yes. Prepare a Master Mix by scaling up the respective component volumes to the desired value. To compensate for pipetting errors, increase the MYtxtl™ reaction volume by at least 4 % of the volume needed, e.g. 100 µL Master Mix for eight 12 µL MYtxtl™ reactions.

3) Are there possibilities to increase the protein production efficiency of the MYtxtl™ reaction?
   Yes. Depending on your application and the protein to be expressed, optimum incubation time and temperature as well as template DNA concentration may vary, and therefore could be evaluated.

4) What is the best device for conducting the incubation of the MYtxtl™ reaction, incubator, thermoblock or water bath?
   We found using a water bath for incubation gives reproducible results, ensuring a constant, fast and reproducible transfer of heat to the reaction tube, although every incubation method should work. Due to the small reaction volume of 12 µL, it is very important to avoid condensation of liquid on lid of the reaction tube, since changes in the reaction volume have a considerable effect on MYtxtl™ performance.

5) Is it possible to freeze a MYtxtl™ reaction after the incubation has been completed?
   Sample handling and storage is mainly determined by the stability of your molecule of interest (protein, DNA, RNA) and thus optimal conditions may need to be evaluated.

6) Is it necessary to measure 10 µL of the same sample in triplicate in the 384-well plate when quantifying the eGFP expressed with MYtxtl™?
   Fluorescence measurement, especially in such small volumes (10 µL) could be susceptible to fluctuation. Therefore, we recommend to measure each sample in triplicate.

7) Do you have any recommendations for measurement settings of the fluorescence reader for the eGFP quantification?
   The most important settings are for the excitation and emission wavelength, which should match the fluorescence properties of eGFP (e.g. $\lambda_{Em.}$ 488 nm, $\lambda_{Ex.}$ 535 nm). Other reader settings such as reading mode, integration time and gain value should be chosen under consideration of high well-to-well fluorescence reading reproducibility.

For any questions and technical assistance, please contact us by phone (+1 (734) 998-0751) or email (techsupport@mycroarray.com). Please visit also our website www.mycroarray.com.