Protocol: myTXTL Sigma 70 Master Mix Kit

The myTXTL Sigma 70 Master Mix Kit is recommended for applications requiring cell-free gene expression from circular (plasmid) DNA templates.

Procedure

The following steps describe setting up myTXTL reactions with the positive control plasmid P70a(2)-deGFP that is supplied with the myTXTL Sigma 70 Master Mix Kit. Please refer to section Options for customization of a myTXTL reaction below for additional information on how to optimize in vitro gene expression for your individual experiment.

1. Preheat incubator (or thermo block or water bath) to 29 °C.
2. Completely thaw the myTXTL Sigma 70 Master Mix and the positive control plasmid P70a(2)-deGFP on ice. Keep reagents on ice till use. Note: To minimize freezing and thawing cycles, only thaw the number of reagent tubes required to set up the desired number of myTXTL reactions.
3. Directly before use, vortex the myTXTL Sigma 70 Master Mix for 2-3 seconds and briefly spin down. If any precipitate is visible hereafter, gently resuspend master mix solution about 10 times to ensure homogeneity. Avoid formation of bubbles and foam.
4. Setting up a myTXTL reaction. The recommended total volume of a myTXTL reaction is 12 µL.

A) Set up a single myTXTL positive control reaction.

On ice, combine components in the order depicted in Table 1 (column 1) in a nucleasefree reaction vessel (e.g. 2 mL Eppendorf tube). Note: Make sure to accurately transfer the entire volume of the positive control plasmid into the Sigma 70 Master Mix by resuspending it 3-4 times. Avoid formation of bubbles. The final concentration of the positive control plasmid is 5 nM.

B) Set up multiple myTXTL positive control reactions at once (replicates).

On ice, combine components in the order depicted in Table 1 (column 2) in a nucleasefree reaction vessel (e.g. 2 mL Eppendorf tube). Note: Simply scale up from volumes needed for a single myTXTL reaction and add at least 4 % of the total volume to account for pipetting errors, e.g. for eight myTXTL reactions (8x 12 = 96 µL) prepare a total volume of 100 µL. Please also read the notes in 4A).

C) Set up a single myTXTL negative control reaction.

On ice, combine components in the order depicted in Table 1 (column 3) in a nucleasefree reaction vessel (e.g. 2 mL Eppendorf tube).

Table 1. Pipetting scheme for setting up myTXTL positive control reactions.

Components	Single myTXTL Positive Control Reaction	Multiple (e.g. Eight) myTXTL Positive Control Reaction	Single myTXTL Negative Control Reaction
Sigma 70 Master Mix	9 µL	75 µL	9 µL
P70a(2)-deGFP Pos. Ctr. Plasmid (20 nM)	3 µL (final: 5 nM)	25 µL (final: 5 nM)	–
Nuclease-free water	–	–	3 µL
Total	12 µL	100 µL	12 µL

5. Gently vortex the reaction mixture for 2-3 seconds and briefly centrifuge the assembled myTXTL reaction to collect the entire volume at the bottom of the tube. The reaction should not contain any bubbles. Place back on ice. In the case of myTXTL reaction replicates: After centrifugation, gently resuspend the mixture 10 times, then split replicate master mix into 12 µL per reaction vessel. Add a final centrifugation step to collect the reaction on the bottom of the vessel.
6. Incubate the myTXTL reaction(s) for up to 16 h at 29 °C.
7. Stop the myTXTL reaction by placing the tube(s) on ice.
8. Evaluate in vitro expression of the gene of interest. The success of the positive control reaction can be evaluated qualitatively (visually) or quantitatively.

Reference

Arbor Biosciences (2019). myTXTL cell-free expression handbook.