PURE*frex*™

Translate your creativity

Description

PURE*frex*™ is the purest cell-free protein synthesis kit based on the PURE system developed by Professor Takuya Ueda. The PURE system only consists of purified factors necessary for transcription, translation and energy regeneration.

Kit content | 250 µL reaction

Reagent	Quantity	Content	Storage
Solution I	125 µL	Amino acids, NTPs, tRNAs, enzyme substrates	-80°C
Solution II	12.5 µL	Proteins in 30 % glycerol buffer	-80°C
Solution III	12.5 µL	Ribosomes [20 µM]	-80°C
Control DNA	10 µL	PCR product containing a gene encoding dihydrofolate reductase [DHFR] from *E.coli* [20 ng/µl]	-80°C

** Please avoid refreeze and thaw as much as possible.*

Step 1 Protocol

Template DNA preparation

PCR products, linear or circular plasmids can be used as the template DNA with the PURE*frex*™ system. We strongly recommend using a sequence optimized for *E.coli* codon usage. Linear templates are also favored over circular ones.

- Please use an intron-free DNA (e.g. cDNA, synthesized DNA).

The template DNA must contain:

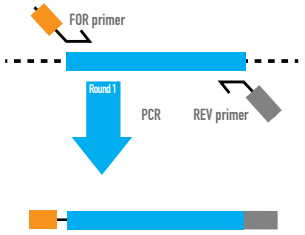
- START codon (ATG)
- STOP codon (TAA, TAG, or TGA)
- T7 promoter (20-100 nucleotides upstream from the coding sequence)
- RBS (prokaryotic ribosome binding site; 6-8 nucleotides upstream from the START)
- More than 10 nucleotides downstream from the STOP
- T7 terminator required for circular DNA template -

T7 terminator sequence:

5'-TAGCATAACCCCTTGGGGCCTCTAAACGGGTCTTGAGGGGTTTTTTG-3'

Example

Linear DNA template generation by overlap PCR.



Round 1

Addition of the regulatory region DNA (RBS, nucleotides downstream STOP codon…) to the 5' and 3' ends of the gene of interest by using gene specific primers (FOR and REV primers).

FOR primer

5'-AAGGAGATATACCA-ATG-N(10-20)-3'

RBS

REV primer

5'-GGATTAGTTATTCA-TTA-N(10-20)-3'.

more than 10 nucleotides of any sequence

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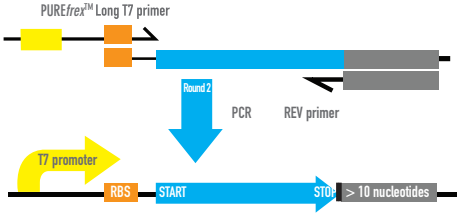

Round 2

Generation of the DNA template containing the gene of interest with complete regulatory sequence and tags by using primers (REV and PURE*frex*™ long T7 primers) and the first round PCR product.

PURE*frex*™ long T7 primer

T7 Promoter

5'-GAAATTAATACGACTCACTATAGGGAGACCACAACG GTTTCCCTCTAGAAATAATTTTGTTTAACTTTAAGAAGGAG ATATACCA-3'.

RBS

This PCR product can be purified or directly used in PURE*frex*™ reaction. Please note that PCR product should be free of non-specific amplification products that can interfere with transcription and/or translation. A tag (e.g. His-tag) can be added to the DNA template to facilitate protein detection and purification.

- We recommend a blue light transilluminator for gel purifying system. UV light sources could lead to reduced transcription efficiencies.
- Using mRNA as template is possible. The mRNA should contain RBS for translation.
- We recommend wearing gloves and using nuclease-free tubes and tips to avoid introducing nucleases to your samples.
- Please use positive control to verify protein synthesis.
- Please use a negative control (without DNA template) to compare the banding pattern.

Step 2 *Protein Synthesis*

1. Thaw Solution I by incubation at 30°C for 1 minute, then cool on ice.
2. Thaw Solutions II and III on ice.
3. Gently vortex each solution separately and centrifuge briefly to collect each solution at the bottom.
4. Assemble the reaction mixture in a tube. The protein synthesis reaction is scalable. For example, 50 µL of reaction is assembled as below:

Water	[20-X] µL
Solution I	25 µL
Solution II	2.5 µL
Solution III	2.5 µL
Template DNA	X µL
Total	50 µL

The optimal DNA amount should be 25-150 ng per 1 kb for a 50 µL PURE*frex*™ reaction (can be determined by setting up multiple reactions). We recommend using 50 ng of positive control per 50 µL reaction.

5. Incubate the tube at 37°C for 2-4 hours. Please note that overnight incubation increases the amount of the produced protein.
6. Analyze the synthesized product (e.g. by SDS-PAGE or activity assay).

Related products & services

Pure*frex*™ long T7 primer	UN-PR500-005	5 nmol
Diamond *Taq*® DNA Polymerase	TAQ-I021-1000	1000 U
Antibody Production Services		
Custom Genes Services		
Custom Oligonucleotides Services		
Anti-tag primary antibodies		

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