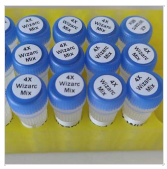

Version 3

May 17, 2021

Cell-free extract, 4x Wizard mix and CFPS reaction preparation- Haseloff Lab V.3

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ABSTRACT

Following this recipe, you will cell-free extracts to perform Tx-TL reactions using plasmid or linear DNA based on P70a and T7 promoters. Likewise, this protocol describes how to prepare 4x wizard mix and setting up a CFPS reaction.

NOTE :

The protocol described here is an adaptation from these papers:

- **Adam D. Silverman**, Nancy Kelley-Loughnane, Julius B. Lucks, and Michael C. Jewett (2019). *Deconstructing Cell-Free Extract Preparation for in Vitro Activation of Transcriptional Genetic Circuitry*. ACS Synthetic Biology, 403-414. DOI: 10.1021/acssynbio.8b00430.
- **Andriy Didovyk**, Taishi Tonooka, Lev Tsimring, and Jeff Hasty. (2017). *Rapid and Scalable Preparation of Bacterial Lysates for Cell-Free Gene Expression*. ACS Synthetic Biology, 2198-2208. DOI: 10.1021/acssynbio.7b00253.
- **Yang WC,** Patel KG, Wong HE, Swartz JR.(2012). *Simplifying and streamlining Escherichia coli-based cell-free protein synthesis*.Biotechnol Prog. 28(2):413-420. DOI:10.1002/btpr.1509.

Please note that some steps were taken literally as they appear in these sources, and it is highly recommended to read these papers before starting.

Our adaptations includes: Changes in buffers, volumes to perform the cell-free reactions using 12 µL as final volume.

ATTACHMENTS

Cell-free Protocol-V1-0.pdf

PROTOCOL CITATION

Fernando FGC Guzman Chavez, Jim Haseloff 2021. Cell-free extract, 4x Wizard mix and CFPS reaction preparation- Haseloff Lab. **protocols.io**
https://protocols.io/view/cell-free-extract-4x-wizard-mix-and-cfps-reaction-bu3xnypn
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KEYWORDS

cell-free, cfps, synthetic biology

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50007

GUIDELINES

To prepare the all solutions described in this protocol see the adequate version from protocol *Work instruction for preparation of CFPS precursor solutions*.

MATERIALS TEXT

- LB medium
- 2XTYG
- 1M IPTG (optional)
- TBST Buffer, pH=7.6
- S30 buffer
- S30 dialysis buffer (optional)
- 2M DTT
- 1000mM Magnesium glutamate
- 10x Salt Solution mix
- 25x Nucleotide mix
- 25x 19 Amino Acid Mix
- 25x PEP
- 40% PEG 8000
- Plasmid or linear DNA purified (20 mM)

Preparing cell extracts

1 5 mL started culture (**BL21 DE Star**) were grown overnight at 37°C from a single colony in LB plate.

2 At the next day 50 mL of LB medium were inoculated with 500 µL of the stationary culture from step 1 and grown at overnight at 37°C.

3 At day 3, 12 mL of the stationary culture were inoculated into 400 mL of 2xYTG and grown at 37°C on an orbital shaker at 200 rpm in a 2.5 L baffled Tunair flask.

4 Culture were grown to the exponential phase optical density (OD600) 2.5-3.0 for approximately 4h. # If you are planning to use plasmid/ linear DNA based on T7 promoter, the culture needs to be induced with **400µL 1 M IPTG** when it reaches an OD of 0.6.

5 Quickly, chill the cells on water/ice bath and incubate on ice for 15 min. #From now on everything has to be performed on ice.

6 Split the culture in two and centrifugate for 12 min at 5000g at 4°C to pellet the cell. # Use 250 mL centrifuge bottles.

7 Wash the 3 times the pellets with each time resuspending them in the buffer: two times with cold TBST and the last with cold S30 buffer supplemented with 2 mM dithiothreitol (DTT). Every time for 12 min at 5000g at 4°C.

8 

Keep and flash frozen the pellet in a pre-weighted 50mL falcon tube. # The pellet can be stored at -80°C from 1 to 2 weeks.

9 Before opening the cells, weight the pellet and resuspend into cold S30 buffer supplemented with 2 mM DTT. #You have to know the weight as you do not want to overdilute the lysate. Rule of thumb is going for 1g of cells/0.9ml of buffer.

10 Open cell quickly with Emulsiflex ~17500-25000 psi and collect the lysate, overall suspension must be passed once. # Wait until pressures changes are stabilized. Alternatively, you can use another cell disruption system as sonication.

10.1 Alternatively, to lysate the cell suspension by sonication, use a QSonica Q125 sonicator with a 3.175 mm diameter probe at a frequency of 20 kHz and 50% amplitude by 10 s ON/OFF pulses for a total of 60 s (delivering~350 J).

11 Transfer the lysate into chilled tubes and centrifuge for 30min at 30 000 g and 4°C, collect 2/3 of the supernatant and repeat the procedure.

12 Then, cell lysate was aliquoted, flash frozen and stored at -80°C.

12.1 ⊛

A) Alternatively, a **runoff reaction** can be performed by transferring the lysate into a falcon tube and incubate at 37°C for 80 min at 200 rpm in dark conditions. Following this incubation, the extract was centrifugated for 10 min at 4°C and 12000g. Then, supernatant was aliquoted, flash frozen and stored at -80°C.

12.2 ⊛

B) Alternatively, a runoff reaction can be performed by transferring the lysate into a falcon tube and incubate at 37°C for 80 min at 200 rpm in dark conditions. Following this incubation, the extract was centrifugated for 10 min at 4°C and 12000g. Then, supernatant was removed and injected into 10K MWCO and **dialyzed** against 600 mL of S30 Dialysis buffer. After, the extract was centrifugated for 10 min at 4°C and 12000g. Supernatant was aliquoted, flash frozen and stored at -80°C.

Preparing 4X Wizard mix

13 To prepare **500 µL of 4X Wizard mix**, thaw the aliquots of each precursor solution and pipette the required volume following the order in the next table.

Precursor solution	Required quantity (µL)
1) Autoclaved MQ water	40
2) 1000mM Magnesium glutamate	20 # for a final concentration of 10mM in CFPS
3) 10x Salt Solution mix	200
4) 25x Nucleotide mix	80
5) 25x 19 Amino Acid Mix	80
6) 25x PEP	80

Vortex the solution so that it is homogeneous. Prepare **aliquots of 50µL**, flash frozen and stored at -80°C.

Performing cell-free reactions

14 To set up a single cell-free reaction of 12µL, thaw the aliquots of each precursor solution and pipette the required volume following the order in the next table. *# Cell-free extract must be added always at the end.*

A	B
Precursor solution	**Required quantity (µL)**
1) Autoclaved MQ water	1.4
2) 40% PEG 8000	0.6
3) DNA (20 nM)	3
4) 4x Wizard mix	3
5) Cell extract	4
Final Volume	12

#40% PEG 8000 should be stored at room temperature. Use microwave to dissolve the PEG 8000, , if it is necessary.

Commentaries

15 To prepare the all solutions described in this file, see the adequate version from protocol " S*olutions for CFPS".*

Literature/ References

16

Silverman AD, Kelley-Loughnane N, Lucks JB, Jewett MC (2019). Deconstructing Cell-Free Extract Preparation for in Vitro Activation of Transcriptional Genetic Circuitry.. ACS synthetic biology. https://doi.org/10.1021/acssynbio.8b00430

Didovyk A, Tonooka T, Tsimring L, Hasty J (2017). Rapid and Scalable Preparation of Bacterial Lysates for Cell-Free Gene Expression.. ACS synthetic biology. https://doi.org/10.1021/acssynbio.7b00253

Yang WC, Patel KG, Wong HE, Swartz JR (2012). Simplifying and streamlining Escherichia coli-based cell-free protein synthesis.. Biotechnology progress. https://doi.org/10.1002/btpr.1509

Change history

17 V1-0 →V1-1 Protocol to cell disruption by sonication was added.
V1-1→ DNA concentration in protocol " Performing cell-free reactions" was corrected