



Jul 11, 2020

2XYT Medium (Version 2-0) Haseloff Lab

Fernando FGC Guzman Chavez¹¹University of Cambridge

1

Works for me

dx.doi.org/10.17504/protocols.io.bigekbteFernando Guzman Chavez
University of Cambridge

ABSTRACT

Work instruction for 2xYT and 2xYTG medium preparation

Following this recipe, you will obtain 1L of 2XYT medium

2X YT is a standard growth medium used to cultivate *E. coli*. In cell-free system, this medium is commonly used to grow the cells.

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.

DOI

dx.doi.org/10.17504/protocols.io.bigekbte

PROTOCOL CITATION

Fernando FGC Guzman Chavez 2020. 2XYT Medium (Version 2-0) Haseloff Lab. **protocols.io**
dx.doi.org/10.17504/protocols.io.bigekbte

KEYWORDS

cell-free, 2XYT, CFPS

LICENSE

————— This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

CREATED

Jul 11, 2020

LAST MODIFIED

Jul 11, 2020

PROTOCOL INTEGER ID

39142

2xYT and 2xYTG medium preparation

For this media, following compounds have to be ready-to-use:

- 2XYT powder
- 1M K_2HPO_4 solution
- 1M KH_2PO_4 solution

1.1 1. Prepare for autoclaving

- Volumes indicated are sufficient for 1 L of **2XYT medium**

Compound, Sum Formula	MW [g/mol]	Required amount [g or mL]	Concentration [g/L]
2X YT powder (16g/L tryptone, 10g/L yeast extract, 5g/L NaCl)	n/a	31 g	31
1M Potassium Phosphate Dibasic [K_2HPO_4]	174.18	40 mL	7[0.04M]
1M Potassium Phosphate Monobasic [KH_2PO_4]	136.09	22 mL	3[0.022M]

Dissolve in demi-H₂O, fill up to 1000 ml, autoclave 15 min at 121°C

2x YTPG Medium

- For 400 mL 2xYTP liquid medium add 7.2g D-glucose (Mw 180.16 g/mol), for a final concentration of 0.1M
- Mix until glucose is dissolved and filter sterilize (0.22µm filter)

3. Literature/ References



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>

4. Change history

Protocol for 2xYTPG medium was included

