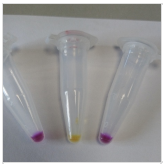

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S30-S30A-S30-Buffers- Haseloff Lab

Fernando FGC Guzman Chavez[1], Jim Haseloff[1]

[1]University of Cambridge

1 Works for me dx.doi.org/10.17504/protocols.io.bigckbsw

Fernando Guzman Chavez
University of Cambridge

ABSTRACT

Following this recipe, you will obtain 1L of **S30**, **S30A** and **S30B Buffers**

S30 Buffer is a standard solution to wash cell pellets previous to lysis. Likewise, it is used during the cell lysis.

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.
- **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.
- https://www.protocols.io/researchers/anibal-arce-medina

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S30 buffer, cell-free

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1. S30 Buffer

1 **To prepare S30 buffer, following compounds have to be ready-to-use:**

- Tris-Acetate
- Magnesium acetate tetrahydrate
- Potassium acetate
- 1M DTT
- 5M KOH

1.1 1. 1 Prepare for autoclaving

- Volumes indicated are sufficient for **1 L of S30 buffer**

Compound, Sum Formula	MW [g/mol]	Required amount [g or mL]	Concentration [mM]
Tris-Acetate	181.19	**1.81g**	10
Magnesium acetate	214.45	**3.0g**	14
Potassium acetate	98.14	**5.89g**	60

Preparation: Dissolve in demi-H2O, fill up to 1000 ml, autoclave 15 min at 121°C
***adjust to pH=8.2 with 5M KOH (approx. 900µL)**

- **Before use** add 2 mL of **1 M DTT** (2mM, final concentration)
- **For dialysis**, S30 buffer is prepared at **5mM** Tris-Acetate and 1mM DTT. Rest of the components remain at same concentrations.
- To prepare **TBST Buffer** (pH=7.6), dissolve 1 tablet of Tris buffered saline with Tween 20 in 500mL of water and autoclave 15 min at 121°C. Catalogue Number: 91414-10TAB SIGMA.

2. S30A and S30B Buffer

2 **To prepare S30A and S30B buffers, following compounds have to be ready-to-use:**

- 100 mM Tris (solution)
- 1:1AceticAcid
- 1M DTT

2.1 2.1 Prepare for autoclaving

- Volumes indicated are sufficient for **1 L of S30 Buffer A and S30 Buffer B**

S30 Buffer A

Compound, Sum Formula	MW [g/mol]	Required amount [g or mL]	Concentration [g/L]
100 mM Tris Base	121.14	**500 mL**	6.057 [50 mM]
Mg-Glutamate	388.61	**5.44g**	5.44 [14mM]
K-Glutamate	203.23	**12.194g**	12.194[60mM]

Dissolve in demi-H2O, fill up to 1000 ml, autoclave 15 min at 121°C
***adjust to pH=7.7 with acetic acid**

- Before use add 2 mL of 1 M DTT

Citation: Fernando FGC Guzman Chavez, Jim Haseloff (07/11/2020). S30-S30A-S30-Buffers- Haseloff Lab. https://dx.doi.org/10.17504/protocols.io.bigckbsw

S30 Buffer B

Compound, Sum Formula	MW [g/mol]	Required amount [g or mL]	Concentration [g/L]
100 mM Tris Base	121.14	**50 mL**	**0.6057 [5 mM]**
Mg-Glutamate	388.61	**5.44g**	5.44[14mM]
K-Glutamate	203.23	**12.194g**	12.194[60mM]

Dissolve in demi-H2O, fill up to 1000 ml, autoclave 15 min at 121°C
***adjust to pH=8.2 with acetic acid**

- Before use add 2 mL of 1 M DTT

3. Literature/ References

3

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

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

- https://www.protocols.io/researchers/anibal-arce-medina

4. Change history

4 V1→V1.1 TBST recipe added.
V1.1→V1.2 Recipes to prepare S30A and S30B buffers were added.

Citation: Fernando FGC Guzman Chavez, Jim Haseloff (07/11/2020). S30-S30A-S30-Buffers- Haseloff Lab. https://dx.doi.org/10.17504/protocols.io.bigckbsw