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1 M Tris-HCl pH 7.5 for *Tetrahymena* mating (Stock solution)

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Protocol status: Working

We use this protocol and it's working

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Keywords: preparation of tetrahymena thermophila cell, tetrahymena mating, tetrahymena thermophila cell, general information about tri, hcl solution, hcl ph, inducing mating behavior, tri, mating, range from ph, mating behavior

Abstract

In this protocol we specify the steps needed to create a 250 ml stock solution of 1M Tris-HCl solution used for the preparation of *Tetrahymena thermophila* cells for mating. The final concentration for inducing mating behavior is 10 mM (see Bruns PJ and Brussard TB, 1974 and Cassidy-Hanley, 2012). For general information about Tris based buffers, check [1](#) [2](#). It buffers in a range from pH 7 to 9.

Citation

Cassidy-Hanley DM (2012)

. Tetrahymena in the laboratory: strain resources, methods for culture, maintenance, and storage. Methods Cell Biol. 2012;109:237-76. .

<https://doi.org/10.1016/B978-0-12-385967-9.00008-6>

LINK

Citation

Bruns PJ, Brussard TB. (1974). Pair formation in tetrahymena pyriformis, an inducible developmental system. J Exp Zool. 1974 Jun;188(3):337-44..

doi: [10.1002/jez.1401880309](https://doi.org/10.1002/jez.1401880309)

LINK

Guidelines

1. As good microbiological practice, all containers used for components should be labeled with the name of the solution, the date of creation / aliquote, and initials of the person who did it, in order to ask questions if something is needed.
2. If you have doubts about how to perform a step in the protocol, consult your supervisor or someone who has prepared the solution before.
3. For any questions, feel free to email us (exotoxinslab-(at)- gmail.com). We are more than happy to help you.
4. Some of the protocols will be posted in our YouTube channel (www.youtube.com/@exotoxinslab).

Materials

-  Tris Puferan >99.3% **Carl Roth Catalog #AE15.3**
-  double distilled water (ddH₂O)
- HCL

Protocol materials

 Tris Puferan >99.3% **Carl Roth Catalog #AE15.3**

 double distilled water (ddH₂O)

Safety warnings

- ❗ The process of autoclaving requires the use of high temperatures and specialized equipment. Make sure you have been instructed in the use of the autoclave.
When handling recently autoclaved liquids, their temperatures can be above 60°C, and can cause severe burns. Follow the safety protocols of your institution and use always personal protective equipment.

In case HCl 37% solution is used, use only under the chemical hood, use lab coat, gloves and goggles.

Also consult the attached data safety sheets:

 tris-safety-data-sheets.pdf 225.9KB

Before start

1. Be aware of your lab's safety protocols and check the attached Warnings (Guidelines and Warnings) to see what steps are necessary to protect yourself, such as protocol-appropriate personal protective gear, like lab coat, gloves and glasses.
2. Make sure to read the whole protocol before starting and have all the materials at hand.



Buffer preparation

35m

- 1 Wash the flask twice with 500 ml of distilled water to remove ANY trace of detergent, which is harmful for *Tetrahymena thermophila*
- 2 In a calibrated cylinder, place 1 magnetic stir bar.
- 3 Weigh  30.28 g of  Tris Puferan >99.3% **Carl Roth Catalog #AE15.3**
- 4 Place the weighted  Tris Puferan >99.3% **Carl Roth Catalog #AE15.3** in the calibrated cylinder and add no more than  200 mL of distilled water.
- 5 Place the cylinder on a magnetic stirrer and dissolve the  Tris Puferan >99.3% **Carl Roth Catalog #AE15.3**.
- 6 While under agitation of the magnetic stirrer, measure the pH. To adjust it to  7.5 , add  1 Molarity (M) HCl drop wise with a pasteur (glass) pipette, all under the chemical hood.
- 7 Add distilled water close to the 250 ml mark. Verify the pH is still  7.5 . Fill to the final 250 ml with distilled water.
- 8 Transfer the solution to the 500 ml pre-cleaned bottle (as described in step 1)
- 9 If the solution needs to to be autoclaved, do so at  121 °C for  00:15:00 to  00:20:00 . Careful! Do not transfer the magnetic stir bar. Make sure you know how to use the autoclave before you use it.
- 10 If the solution does not need to be autoclaved, transfer the 250 ml to a 250 ml pre-washed (see step 1) flask and stored at  Room temperature .

35m





Protocol references

<https://www.dcfinechemicals.com/en/blog/trishydroxymethylaminomethane-tris-buffer/> (accessed November 2024)

<https://sigmaaldrich.com/DE/en/products/chemistry-and-biochemicals/biochemicals/biological-buffers/tris> (accessed November 2024)

Bruns PJ, Brussard TB. Pair formation in tetrahymena pyriformis, an inducible developmental system. J Exp Zool. 1974 Jun;188(3):337-44. doi: 10.1002/jez.1401880309. PMID: 4209195.

Cassidy-Hanley DM. Tetrahymena in the laboratory: strain resources, methods for culture, maintenance, and storage. Methods Cell Biol. 2012;109:237-76. doi: 10.1016/B978-0-12-385967-9.00008-6. PMID: 22444147; PMCID: PMC3608402.

Citations

Bruns PJ, Brussard TB.. Pair formation in tetrahymena pyriformis, an inducible developmental system.
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Cassidy-Hanley DM. Tetrahymena in the laboratory: strain resources, methods for culture, maintenance, and storage.
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