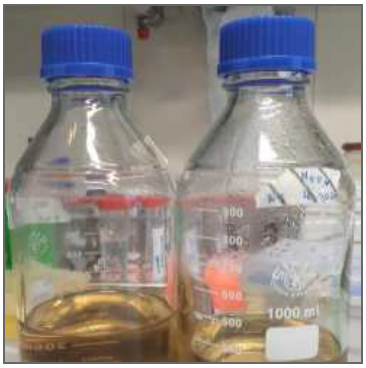

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Protocol status: Working
We use this protocol and it's working for at least 70 passages of T. thermophila

Created: May 03, 2023

Veggie NEFF media for Tetrahymena thermophila

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ABSTRACT

The ciliate *Tetrahymena thermophila* has been a research organism used by a great community of scientists including biologist, geneticists and toxicologists. *T. thermophila* is a bacterial predator; however, we can grow it in the lab without bacteria, in axenic media, if needed.
The media presented here is a modification of the axenic NEF media published in the Tetrahymena stock center (RRID:SCR_008362, https://tetrahymena.vet.cornell.edu/recipes.php) under modified NEF media, and originally published by Cassidy-Hanley D, Bowen J, Lee JH, Cole E, VerPlank LA, Gaertig J, Gorovsky MA, Bruns PJ. Germline and somatic transformation of mating Tetrahymena thermophila by particle bombardment. Genetics. 1997 May;146(1):135-47. doi: 10.1093/genetics/146.1.135. PMID: 9136007; PMCID: PMC1207932.
We have modified the media to use peptone free from animal products. The original component "Proteose Peptone" is the processed result of animal products, which brings high variability associated with each production lot every time we buy a new product. Because of this, we have been growing cells and testing some behaviors while using peptone from soy. This is the media we present here.
The protocol has three sections: First part describes how to prepare NEFv (NEF media with "vegetarian" peptone from soy and without iron complement), the second describes how to prepare the stock concentration of iron chloride and its storage, and the third describes how we get all components together just before we use the media.

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PROTOCOL integer ID: 81316

GUIDELINES

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.
If you have doubts about how to perform a step in the protocol, consult your supervisor or someone who has prepare the solution before.

PROTOCOL MATERIALS

- Iron(III) chloride hexahydrate 250 g **Carl Roth Catalog #P742.1** Step 10
- Yeast Extract 250 g **Carl Roth Catalog # 2363.1** Step 3
- D( )-Glucose monohydrate **Carl Roth Catalog #6887.1** Step 3
- Peptone ex soya (animal-free/GMO-free) 250 g **Carl Roth Catalog #2832.1** Step 3

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.

BEFORE START INSTRUCTIONS

Make sure that you have all materials, you know who to work with all equipment needed, and you are using your personal protective (PP) equipment during work.

Section 1: NEF veggie media

4h

1 1m

Prepare 4 bottles of 500 ml volume each. Make sure the bottles are free of detergent rests, by washing at least 4 times with distilled water. Label each bottle with the name of the media: NEFv (NEF veggie)

Equipment

Screw top bottle, clear glass. Volume 500 ml	NAME
Glass material	TYPE
ROTILABO	BRAND
X714.1	SKU
https://www.carlroth.com/com/en/glass-bottles/screw-top-bottle-rotilabo-clear-glass/p/x714.1	LINK
Borosilicate glass bottle , with GL45 thread and plastic cap. Autoclavable. Volume 500 ml	SPECIFICATIONS

2 In a 1 liter graduated cylinder, add a magnet stirrer. 30s

3 Weight the following components and deposit them inside the graduated cylinder: 4m

- 2.5 g Peptone from Soy
- 2.5 g Yeast extract
- 5 g D(+) Glucose Monohydrate

Do not forget to clean the area where weighting took place. Peptone and yeast extract produce a considerable amount of dust while handling, and if left uncleaned, the scale can be damaged (not to mention your relationship with your colleagues)

Peptone ex soya (animal-free/GMO-free) 250 g **Carl Roth Catalog #2832.1**

Yeast Extract 250 g **Carl Roth Catalog # 2363.1**

D(+)-Glucose monohydrate **Carl Roth Catalog #6887.1**

4 Add distilled water to the graduate cylinder containing the magnet stirrer and the components weighted in step 3, to a final volume of 1 liter. Place the cylinder onto a magnetic plate and stir the solution until all components have dissolved. You should obtain a clear and slight yellow solution. 5m

5 Once dissolved, pour 250 ml of the solution into each of the labelled bottles (prepared in step 1) . 1m

6 Close the bottles and turn their cap about half of a turn back. The bottle should now be slightly open. Use the sterilization tape to make sure that the cap will not move. Achieve this by placing part of the tape onto the cap surface and the other onto the bottle side. This guarantees that the cap will remain on the bottle, and it will prevent the explosion of the bottles during the wet sterilization process. 5m

7 Sterilize the media using an autoclave. The program for liquids should not be shorter than 15 min but n 2h 30m longer than 20 minutes. This allows a good sterilization and reduces the caramelization of the glucose, and the Maillard reaction in the amino acid components in the media.

8 Once the autoclave indicates the cycle has ended, and that it is safe to open, remove the bottles from it **and** 5m **close them tight immediately** to avoid contamination. Careful! The bottles are extremely hot (above 60 °C). Use personal protective equipment to avoid injuries.

9 You can now store the media at room temperature, protected from light, until you would like to use it. 30w

Section 2: 33 mM Iron Chloride stock solution 50m

10 Weight 0.446 g of $FeCl_3$. $6H_2O$ and place it in a 50 ml conical tubes. 5m

Iron(III) chloride hexahydrate 250 g **Carl Roth Catalog #P742.1**

11 Add distilled water up to the 50 ml marking (concave liquid limit bottom on the 50 ml marking) 30s

12 Dissolve the salt particles until they disappear. This may take some time. If a roller is available, place the 50 ml conical tube on it for mixing. 5m

13 Only after all particles have disappeared, filter the solution through the 0.22 µm pore filter into a new sterile conical tube. 2m

14 Make four 10 ml aliquotes in 15 ml sterile conical tubes, properly labelled, and store at -20°C. With the left ml (usually 1 ml is lost during filtration), aliquote them in 1,5 ml tubes, each with 500 µl only and store in -20 °C. Make sure you have labelled the tubes: on top and side with the name of the solution, and on the side write the concentration, date and your initials. One aliquote of 500 µl can be stored at room temperature, protected from light, for about 4 - 5 months. **Discard as soon as the plastic of the tube shows signs of iron deposits on the walls (yellow tint), or precipitated brown particles are visible at the bottom of the tube**. When thawing, make sure that the precipitated particles are dissolved at room temperature before using the solution. 35m

Section 3: NEFFv - Getting the media ready for use

2m

15 The description here is for preparing fresh 50 ml of veggie NEFF (NEFFv) media for use in culture of *T. thermophila* . You can scale it up as needed. **Under sterile conditions**, take 50 ml of NEFv media (section 1) in a 50 ml conical tube. 1m

16 Add 50 µl of the 33 mM Iron chloride solution for a final concentration of 33 µM $FeCl_3$ to create the NEFFv. Mix thoroughly. Media is ready to use. **You can store the NEFFv for up to two weeks at room temperature**. After that time, cells do not seem to grow the same. 1m