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Version 2

Prepare Tris Buffer for Meat identification Protocol V.2

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Protocol status: In development

We are still developing and optimizing this protocol

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Abstract

Buffer protocol for meat identification experiment

Materials

reagents: Tris, HCl (12.1M), dd-di-DNAase free water. Note: the dilution of HCL can also be 1M.

devices: scale, thermometer optional: incubator, magnetic stirrer or glass rod for stirring, pipettes, pipette rack, tube rack, pen

labware: 50 ml beaker large enough to permit pH meter measurements

consumables: 50 ml falcon tube

Protocol materials

 Tris **Bio Basic Inc. Catalog #TB0196**

 Hydrochloric Acid **Bioshop Catalog #HCL333.500**

Before start

Make sure you adjust the pH to the same temperature for the reaction in which the buffer is used.

Prepare Tris Buffer for Meat identification Protocol. Prepare (50 ml) of 0.04 mol/L Tris HCl (pH 7.75)

1 Add  25 mL of deionized DNase free, distilled-water to a 50 ml beaker.

2 Measure and add  0.24 g of  Tris Bio Basic Inc. Catalog #TB0196

Calculation for  Tris Bio Basic Inc. Catalog #TB0196 weight:

MW for TRIS (C₄H₁₁NO₃) 121.14 mol/Liter

0.04 M TRIS TRIS : $m = CMV = 0.04 \text{ mol/L} * 121.14 \text{ g/mol} * 50 \text{ ml} = 0.24 \text{ g}$ of TRIS

<https://www.cytographica.com/lab/HHTris.html>

3 Add  100 μ L HCl [M] 12.1 Molarity (M)

 Hydrochloric Acid Bioshop Catalog #HCL333.500

4 Bring the beaker to final experiment desired Celsius temperature ( 25 °C Celsius for example) and maintain. Can use a water bath or an incubator for example.

5 Add about  100 μ L HCl [M] 12.1 Molarity (M) then iteratively measure pH and add 1 to 2 μ L HCL until pH reaches  7.75 . The calculation below indicates that about 116 μ L in total should achieve the desired pH.

<https://www.cytographica.com/lab/HHTris.html>

!! All this steps are done at the desired temperature set in the previous step. That is required because any pH measurement has to be done at a set temperature as pH is temperature dependent.

Note

The pH indicated in the referenced paper is  7.75 compared to  7 in the main paper.

**Note**

The initial pH should be high around 10 or 11 as Tris is alkaline and should decrease with the addition of HCl.

- 6 Transfer from beaker to a 50 ml falcon tube for storage.
- 7 Add water to bring the total to  50 mL .
- 8 Store the falcon tube in the fridge for max 4 months.

Protocol references

Normally we make half the solution and titrate with HCl

[https://www.researchgate.net/post/How-to-make-a-Tris-HCl-buffer#:~:text=Tris%2DHCl%20can%20be%20prepared,is%20157.6%20g%2Fmol\).](https://www.researchgate.net/post/How-to-make-a-Tris-HCl-buffer#:~:text=Tris%2DHCl%20can%20be%20prepared,is%20157.6%20g%2Fmol).)

pH calculations= <https://www.youtube.com/watch?v=387t-nh0OMg>

<https://www.cusabio.com/m-296.html#a21> for calculating Tris HCL pH from Tris and Hydrochloric acid

https://www.reddit.com/r/labrats/comments/193ei6n/trying_to_prepare_trishcl_buffer_ph_seems/?rdt=42146