Conventional fixation method for *Tetrahymena thermophila* V.1

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Protocols for studying Tetrahymena meiosis

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A rapid and robust method for fixing Tetrahymena thermophila cells

miao.tian 2022. Conventional fixation method for Tetrahymena thermophila. **protocols.io** https://protocols.io/view/conventional-fixation-method-for-tetrahymena-therm-b45cqy2w

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General description of the method

1 It works for the IF of cytoplasm, nucleoplasm, and chromatin-bound proteins, FISH staining of receptive sequences (e.g., telomeric repeats, TEs). Nuclear and cell morphology are nicely maintained.

It **does not** work for the IF of Cna1, γ-H2A.X, and tubulin.

Reagents

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- 37% Formaldehyde (! Toxic, handel it in a fume hood)
- Triton X-100
- Sucrose
- Paraformaldehyde (!Toxic; Cat# A3813, 1000. Applichem)

Recipes for making solutions 17h

3 Preparing 4% formaldehyde/ 3.4% sucrose fixative **100 mL**

3.1 Weight 4 g of Paraformaldehyde, add **100 mL** of water. 55m

- Heat to dissolve Paraformaldehyde in a fume hood. Namely, placing the flask on top of a **170 °C** heat plate with stirring function, for around **00:25:00** , with stirring. Try not let it boil.
- Leave it on bench to cool for around **00:30:00** (do not make it completely cool, otherwise sugar dissolves very slow).

3.2 Add 3.4 g of Sugar into the warm paraformaldehyde solution, stir/dissolve it. 16h

- leave it at room temperature overnight and then store it in a fridge.

4 10% Triton X-100 **50 mL**

- Add **5 mL** of Triton X-100 into **45 mL** of water. Microwave for 5s will let Triton X-100 dissolve rapidly.

Fixation of 5 mL of *Tetrahymena* cells

5 (!) For growing cells: Wash cells once with **10 millimolar (mM)** Tris-HCl (pH **7.5**) and resuspend cells with the same volume of **10 millimolar (mM)** Tris-HCl (pH **7.5**). Starvation or conjugation cells need not to be washed for another time.

6 Shoot **250 µL** of 10% Triton X-100 (first) and **500 µL** of 37% formaldehyde into cells. 30m
Invert tube ~ 5 times and keep it at room temperature (**25 °C**) for **00:30:00** ;

7 Collect cells by centrifugation at 1500 rpm for **00:01:00** and pour supernatant (! contains formaldehyde, think about waste disposal rules) 1m

8 Resuspend cell pellet with ~ **500 µL** of 4% formaldehyde/ 3.4% sucrose fixative, mixing cells by gentle pipetting is recommended.

9 Cells are ready to apply onto slides. After drying in a fume hood for 1hr, slides are ready for staining.