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Conventional fixation method for *Tetrahymena thermophila* V.1

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protocol .

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

- 2
 - 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 μ L of 10% Triton X-100 (first) and  500 μ L 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^{1m} formaldehyde, think about waste disposal rules)
- 8 Resuspend cell pellet with ~  500 μ L 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.