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Conventional fixation method for *Tetrahymena thermophila* V.2

miao.tian¹¹Ocean University of China

protocol .

Protocols for studying Tetrahymena meiosis

miao.tian

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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-b45fqy3n>
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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

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

Fixation of 5 mL of *Tetrahymena* cells

31m

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

6 Shoot of 10% Triton X-100 (first) and of 37% formaldehyde into cells.^{30m}
Invert tube ~ 5 times and keep it at room temperature () for ;

7 Collect cells by centrifugation at 1500 rpm for and pour supernatant (! contains^{1m} formaldehyde, think about waste disposal rules)

8 Resuspend cell pellet with ~ 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.