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Method Article

# A simple and fast method for fixation of cultured cell lines that preserves cellular structures containing gamma-tubulin

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A B S T R A C T

When using fluorescence microscope techniques to study cells, it is essential that the cell structure and contents are preserved after preparation of the samples, and that the preparation method employed does not create artefacts that can be perceived as cellular structure/components. γ-Tubulin forms filaments that in some cases are immunostained with an anti-γ-tubulin antibody, but this immunostaining is not reproducible [1]. In addition, the C terminal region of γ-tubulin (green fluorescence protein tagged [GFP]-γ-tubulin$^{334-449}$) forms cytosolic GFP-labeled structures, which can easily be imaged in live cells but are not preserved in fixed cells [1,2]. The purpose of this study was to identify a fixation technique that preserves cellular constituents containing γ-tubulin.

- This protocol describes a method that preserves γ-tubulin-containing structures in fixed cells.
- The technique entails two-step fixation. A pre-fixation step using paraformaldehyde is followed by a final fixation and permeabilization step performed at −80 °C.
- In comparison with other methodology for fixation [3–5], the technique presented here uses a short pre-fixation step with a mixture of paraformaldehyde and sucrose followed by a short fixation/permeabilization step with a mixture of methanol and acetone at −80 °C.



A R T I C L E I N F O

*Method name:* Fixation technique

*Keywords:* Immunofluorescence, Cell lines, Cytoskeleton

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## Specifications table

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## Method details

Live imaging followed by fixation of the imaged cells

*Step 1: preparation of stable cell lines*

Materials

- U2OS human osteosarcoma cells (ATCC®HTB-96™)
- 35-mm tissue culture dishes
- Jet Pei (Polyplus Transfection, cat. no. 101-10N)
- pEGFP-γ-tubulin$^{334-449}$ [6] (Addgene Plasmid # 87859)
- pTER-*γTUBULIN* shRNA [7] (Addgene Plasmid # 87955)
- 35-mm MatTek glass bottom dishes (MatTek corporation, cat. no. P35G-0.170-14-C)
- Zeocin (ThermoFisher Scientific, cat. no. R25001)
- Geneticin (G418; ThermoFisher Scientific, cat. no. 10131035)

Note that this list includes only necessary cell lines and non-standard laboratory equipment.

1. In a 35-mm tissue culture dish, U2OS cells are cultured and transfected at a ratio of 1:1 of pTER-*γTUBULIN* shRNA and pEGFP-γ-tubulin$^{334-449}$ using Jet Pei according to the instructions of the cells distributors and the Jet Pei manufacturer.
2. Two days after transfection, stably transfected U2OS cells co-expressing *γTUBULIN* shRNA (reduces the expression of the endogenous γ-tubulin pool) and human GFP-tagged sh-resistant γ-tubulin C termini (GFP-γ-tubulin$^{334-449}$), designated *γTUBULIN*sh-U2OS–GFP-γ-tubulin$^{334-449}$ cells, are obtained by supplementing the cell medium with both 100 μg/mL zeocin and 200 μg/mL G418. Non-transfected cells will die within 2–3 days.
3. Add fresh medium supplemented with 100 μg/mL zeocin and 200 μg/mL G418 as deemed suitable. Passages to new cell culture dishes are not necessary until the 35-mm tissue culture dish is filled to capacity. It takes 2–3 weeks to obtain a stable cell line.
4. Once a stable *γTUBULIN*sh-U2OS–GFP-γ-tubulin$^{334-449}$ cell line is established, plate $3 \times 10^5$ *γTUBULIN*sh-U2OS–GFP-γ-tubulin$^{334-449}$ cells on a 35-mm MatTek glass bottom dish 24 h before imaging.

*Step 2: cell imaging of live and fixed cells*

Materials

- Phosphate-buffered saline (PBS)
- Freshly made 4% paraformaldehyde and 2% sucrose dissolved in PBS (Psuc buffer); this solution can be stored at −20 °C for approximately 2–3 weeks
- 1:1 methanol, acetone solution (Met/Ac)
- A felt-tip pen
- A fluorescence/confocal microscope with a cell incubator equipped with a heating and $CO_2$ system (necessary for incubations longer than 3 h)