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Labeled microtubules for single-molecule imaging

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This protocol is for making labeled taxol-stabilized microtubles to be used for single-molecule imaging assays by adhering to biotin slides.

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Required Buffers:

5x BRB80:

- [M]**400 millimolar (mM) PIPES, pH 6.8 with KOH**
- [M]**10 millimolar (mM) MgCl2**
- [M]**5 millimolar (mM) EGTA**

2x Polymerization Mix:

- [M]**2 x BRB80**
- [M]**2 millimolar (mM) DTT**
- [M]**2 millimolar (mM) GTP (Add last)**
- [M]**2 millimolar (mM) MgCl2 (Add second to last)**
- [M]**20 % DMSO**
- Mix well between adding each ingredient

For hazard information and safety warnings, please refer to the SDS (Safety Data Sheet).

Please take notice of the buffer preparation in the Materials section

Create taxol-stabilized labeled microtubules (can be reused for multiple weeks): 50m

1

In a prechilled 1.5 mL Eppendorf tube, make a **10 µL** mixture of [M]**10 mg/mL tubulin** .
The mixture should be 80% unlabeled, 10% biotin-tubulin, and 10% 405-tubulin.
Mix by gently flicking.

2 10m

Let it sit **On ice** for **00:10:00** .

3

Add equal volume of [M]**2 x polymerization buffer** (**10 µL**). Mix by gently flicking.

4 30m

Incubate at **37 °C** for **00:30:00** . Make a [M]**1 x BRB80** + [M]**1 millimolar (mM) DTT**

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+ [M]**20 micromolar (µM) Taxol** stock and incubate at **37 °C** at the same time.

5 

Add equal volume of prewarmed [M]**1 x BRB80** + DTT + Taxol (**20 µL**). Mix by gently flicking.

6 

10m

Incubate at **37 °C** for at least **00:10:00** (solution will be stable for hours at this point).

7 Store in the dark at **Room temperature** . Should be usable for several weeks, but more aggregates will appear over time.

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