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Labeled microtubules for single-molecule imaging

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Protocol status: Working
We use this protocol and it's working

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Abstract

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

Materials

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

Safety warnings

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

Before start

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

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

4 Incubate at 37 °C for 00:30:00 . Make a [M] 1 x BRB80 + [M] 1 millimolar (mM) DTT + [M] 20 micromolar (µM) Taxol stock and incubate at 37 °C at the same time. 30m

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

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

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