Microtubule Spindowns for Visual Analysis

Microtubule spindowns for visual analysis can be performed on single microtubules or microtubules nucleated from axonemes/centrosomes. Although live DIC analysis has largely superseded the use of visual fixed time point assays for analyzing microtubule dynamics, spindowns can still be very useful. Procedures for fixing and pelleting microtubules onto coverslips are described here.

I. Solutions & Supplies

BRB80 (1X): 80 mM PIPES, 1 mM $MgCl_2$, 1 mM EGTA, pH 6.8 with KOH (generally made as a 5X stock and stored at 4iC)

Fix: 1% glutaraldehyde in BRB80 (prepare from a 50% glutaraldehyde stock just prior to use)

Cushion: BRB80 + 10% (v/v) glycerol

Spindown tubes with 12 mm coverslips (Evans et al. J. Cell Biol., 1985)

HB-4/HB-6 or equivalent rotor

A common problem with spindowns is guessing the right volume to sediment onto a 12 mm diameter coverslip. This will require some amount of practice/troubleshooting. With stable microtubules (taxol- or GMPCPP-stabilized), use squashes to guess what volume to spin down. Most of the time, people err on the side of excess -- e.g., it only takes ~0.2 µl of 1 µM CPP microtubules to get a good density of single microtubules on a 12 mm diameter circle coverslip.

II. Spindown Protocol

1. Setup spindown tubes with chocks (plexiglass inserts) and coverslips and add 5 ml BRB80. Then use a very cutoff blue tip to underlay the BRB80 with ~2 ml of Cushion (BRB80 + 10% glycerol). Make sure the tubes are balanced and store in an ice bucket.

2. Fix the reaction containing microtubules by adding 10 volumes of Fix and mixing gently with a cutoff tip. Incubate at RT for 3'.

3. Dilute fixed reaction with 20-30 volumes of BRB80, mix by inversion and store on ice until all samples have been processed.

4. Pipet the appropriate amount of fixed and diluted reaction on top of the BRB80 in the spindown tubes. Spin at 12,500 rpm in an HB-4/6 rotor at 20iC for 1-1.5 hours (start with rotor and centrifuge at 4iC; the centrifuge will heat up to 20iC very rapidly and this will save some centrifuge wear & tear)

5. Aspirate and postfix in -20iC methanol for 5'. Rehydrate and either mount (if microtubules are fluorescent) or perform indirect immunofluorescence.

The same procedure can be used with microtubules nucleated off axonemes or centrosomes. For pelleting these larger structures, use a 5 ml 30% (v/v) glycerol cushion and spin at 10K for 15'-20' at 20iC. Wash the sample cushion interface well prior to aspirating and processing the coverslips.