

## Spindle Assembly in *Xenopus* Egg Extracts

This protocol is essentially as described by Sawin and Mitchison “*Mitotic spindle assembly by two different pathways in vitro*“, J Cell Biol. 1991 Mar;112(5):925-40.

Freshly prepare CSF extract.

Keep extract on ice.

Per 20  $\mu$ l CSF extract, add

- labeled tubulin (1:50 – 1:200 depending on labeling ratio)
- 100 sperm nuclei/ $\mu$ l extract
- 0.6 mM CaCl<sub>2</sub>

Incubate at 16°C for 90 min.

Add back 0.5 Vol CSF-extract.

Incubate another 45 min.

Simply fix samples by spotting 3-4  $\mu$ l of extract reaction onto a coverslip and overlay with 3-4  $\mu$ l fix solution<sup>1</sup> and cover glass. Analyse by fluorescence microscopy.

### (1) Fix solution

0.3 ml formaldehyde 37%

0.6 ml glycerol 80%

1 ml 1x MMR

1  $\mu$ l Hoechst No. 33342 (10 mg/ml)