

Immunofluorescence protocol for TPX2 staining.

TPX2 is a factor implicated in regulation of the mitotic spindle microtubules and poles. Human TPX2 can be easily visualized in mitotic cells using Ab NB500-179 under standard immunofluorescence conditions:

- HeLa were cells grown on sterile coverslips and fixed in cold methanol (-20°C) for 6 minutes.
- Blocking steps and antibody incubations (1 hour) were carried out in PBS/0.05% Tween-20 containing 3% BSA at room T°.
- After blocking, the slides were incubated with TPX2 (Novus NB500-179), used at 1:2000 dilution, and an alpha-tubulin antibody to visualize the mitotic spindle (1 hour, room T°).
- After incubation with primary antibodies, the slides were briefly washed and further incubated with species-specific anti-IgG antibodies (for TPX2, Cy 3-conjugated; for alpha-tubulin, FITC-conjugated). The chromosomal DNA was stained with 4,6-diamidino-2-phenylindole (DAPI, 0.1 µg/ml, 10 min).
- The slides were finally mounted in Vectashield and observed under an epifluorescence microscope.

References

[Importin-β negatively regulates multiple aspects of mitosis including RANGAP1 recruitment to kinetochores.](#)

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[Control of Aurora-A stability through interaction with TPX2.](#) Giubettini M, Asteriti IA, Scrofani J, De Luca M, Lindon C, Lavia P, Guarguaglini G. J Cell Sci.;124(Pt 1):113-22. doi: 10.1242/jcs.075457.