CENP-F IMMUNOFLUORESCENCE (IF) PROTOCOL

CENP-F has a complex localization pattern in human mitotic cells: it is targeted to both the mitotic spindle microtubules and to chromosomal kinetochores by specific partners with which it associates during mitosis. In metaphase, CENP-F is particularly enriched at the outer kinetochores of chromosomes.

1. To obtain high resolution images of kinetochore-associated proteins, HeLa cells grown on sterile coverslips are subjected to mild pre-extraction in 0.005% digitonin in MEKH buffer (2 mM $Mg(OAc)_2$; 0.5 mM EGTA; 110 mM KOAc; 20 mM Hepes) freshly supplemented with 2 mM DTT and 1 µg/ml protease inhibitor cocktail (leupeptin, pepstatin and aprotinin).
2. Cells are then fixed for 15 minutes at room temperature in 3.7% paraformaldehyde in MEKH buffer.
3. Cells are blocked in 3% BSA/0.05% Tween-20 in PBS, then incubated for 1 hour at room T° with CENP-F (NB500-101, Novus) diluted 1:200 in 3% BSA/0.05% Tween-20 in PBS.
4. After washing, anti-rabbit Cy3-conjugated secondary antibody is added and incubation is continued for 30 min. Chromosomal DNA is stained with 0.1 µg/ml DAPI.
5. Coverslips are finally mounted in mounting medium.

Reference

Importin-β negatively regulates multiple aspects of mitosis including RANGAP1 recruitment to kinetochores. Roscioli E, Di Francesco L, Bolognesi A, Giubettini M, Orlando S, Harel A, Schininà ME, Lavia P. J Cell Biol. 2012;196(4):435-50. doi: 10.1083/jcb.201109104.