



INCELLIS®: PERFECT TOOL TO QUALIFY THE CELL LABELLING BEFORE USING WITH A HIGH END MICROSCOPE

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/ CONTEXT

Centrosome is an organelle implicated in cell cycle regulation. During cell division, the two centrosomes serve as anchors to guide the chromosomes and divide them into two equitable batches to form the two daughter cells. An aberrant numbers of centrosomes in a cell is often associated with cancer.

Expression of GFP-centrin recombinant protein into in vitro cell culture allows direct visualization of centriole behavior in living cells in different experimental conditions. Nether the less, as it is a small organelle, imaging of GFP-centrin protein into centrosome requires high sensitivity in fluorescence.

InCellis®, thanks to its low light sensor provides high quality images to easily control, directly on the bench, the quality of centrosome labeling before the analysis with a high end microscope.

/ MATERIALS

- InCellis® Cell Imager for Brightfield, Phase contrast and Fluorescent applications 004393-003-RD0001-A
- InCellis® - 40X FL/Ph LWD objectives: for petri dish, flask
- InCellis® GFP F.L.M.
- InCellis® RFP F.L.M.
- Stable transfected HeLa cell line (supplied by UMR INSERM1052)

/ PROTOCOL

To control the quality of a frozen stable HeLa cell line transfected with Centrin 1 (GFP) and Nucleosine (mCherry), we inoculate an aliquot of cells for 24 h at 37°C into a T-25 flask. On day 2, the culture is visualised to check the fluorescence and the viability of the cells.

The centrosome is imaged with the GFP Fluo Light Module and the nucleolus is imaged with RFP Fluo Light Module.

The growth of the cells is controlled using the white light channel.

/ CONCLUSION

InCellis® and its unique Low Light CMOS sensor give a tight control of the quality of cell culture labelling before observation on confocal microscope. Viability, fluorescence, transfection, observation of very small organelles (less than 1 µm) as well as larger one (2-4 µm), are critical factors in successful cell imaging experiments. The Centrin 1 GFP is easily detected directly on the bench and show that the cell line expressed the Centrin 1 and Nucleosine as expected. The experiment demonstrate that InCellis® brings a high level of sensitivity and allows to validated the quality of sample before using it for time consuming and expensive downstream experiments.

/ RESULTS

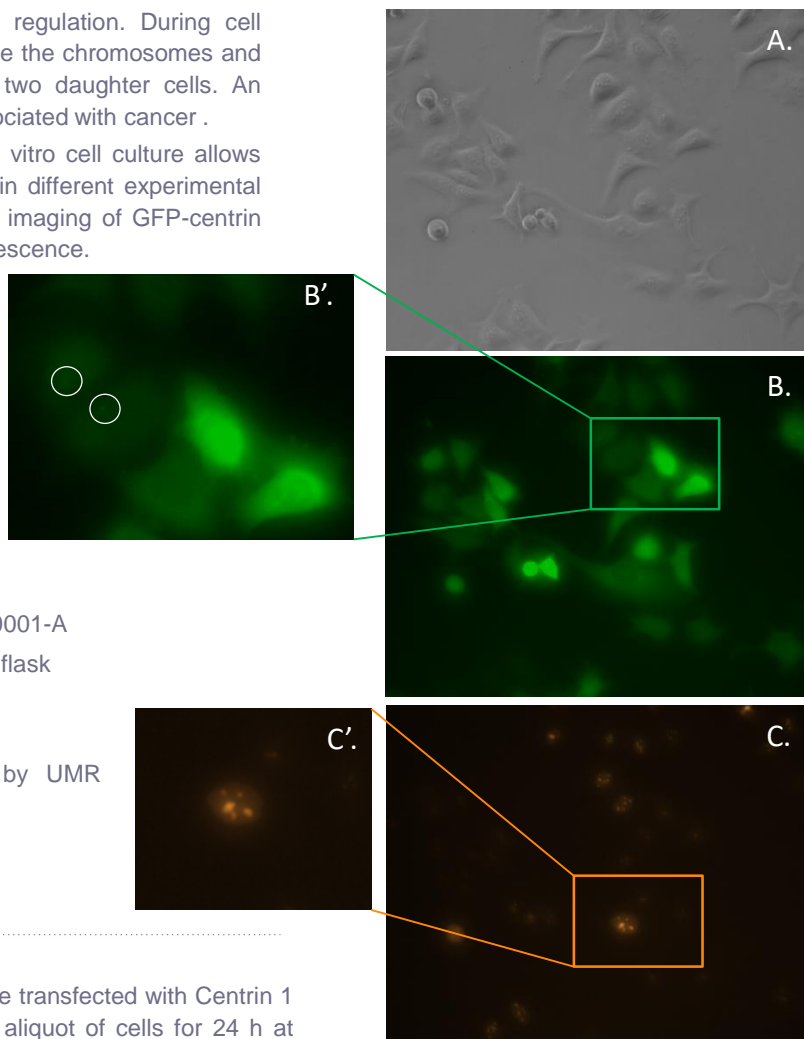


Fig1: A. Image in Phase contrast to control cell growth

B. Image of GFP labelling to control the stable transfection of Centrin – visualisation of the centrosome (B')

C. Image RFP labelling to control the stable transfection of Nucleosine - visualisation of the nucleolus (C')

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