In Vitro Application

APPLICATION NOTE:

Immunofluorescent Staining of Actin, Tubulin, and Nuclei in PANC-1 and HEK293 Cells Cultured in 96-well LifeGel Plates.

Materials required:

- PANC-1 and HEK293 cells 3D culture on 96- well LifeGel Plates (see protocol: Preparing LifeGel Plates)
- 4% PFA
- PBS
- 0.1% Triton X-100
- 10% FBS
- Antibodies
- Pipette
- Fluorescent microscope

STAINING PROTOCOL:

- Fix cells using 4% formaldehyde (10% formalin solution) – 100 µL per well and incubate for 15-30 minutes at room temperature (the bigger 3D structures, the longer time of incubation is recommended).
- Wash cells 3x with 150 µL of PBS.
- Perforate cells using 0,1% Triton X-100 in PBS with 10% FBS. Use 100 µL of solution per well and incubate for 15-30 minutes at room temperature (time similar as for cell fixation).
- Wash cells twice with PBS.
- Change PBS for 100 µL of working solution of primary antibody (1:500 dilution) and incubate plate overnight at 4ºC.
- Next day wash cells 3 times with PBS (wait 5 minutes between each washing).
- Prepare mix of working solution containing secondary antibody (1:250), phalloidin (1:500) and Hoechst (10 µg/mL) in PBS. Add 100 µL per well and incubate plate for 3 hours at room temperature in darkness.
- Wash cells with PBS, 3 times (wait 5 minutes between each washing).
- Then take images of brightfield and fluorescence using fluorescence microscope and objectives characterized by working distance not less than 2 mm – objectives with higher magnifications like 10x and 20x are preferable for cytoskeleton imaging.

REAL RESEARCH

Real Research S.A.
Jagiellonian Center of Innovation
Prof. M. Bobrzyńskiego 14
30-348 Kraków, PL

The Bradfield Centre
184 Cambridge Science Park
Milton Road,
Cambridge, CB4 0GA, UK

In Vitro Application

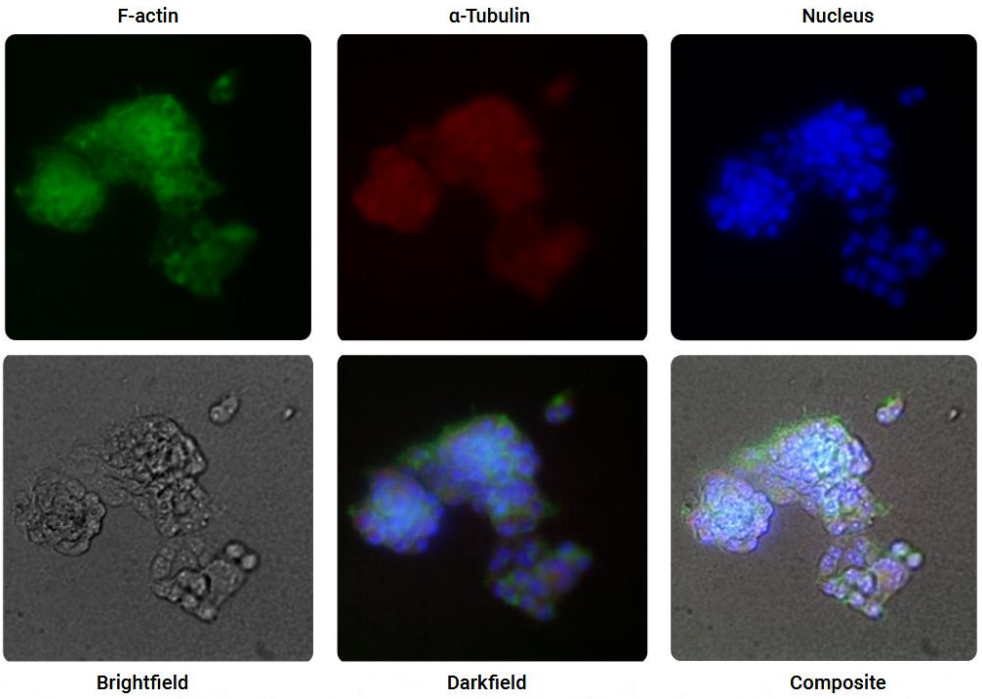

Fig. 1. PANC-1 spheroids immunostaining on LifeGel. Objective 10x, optovar lenx 2,5x.

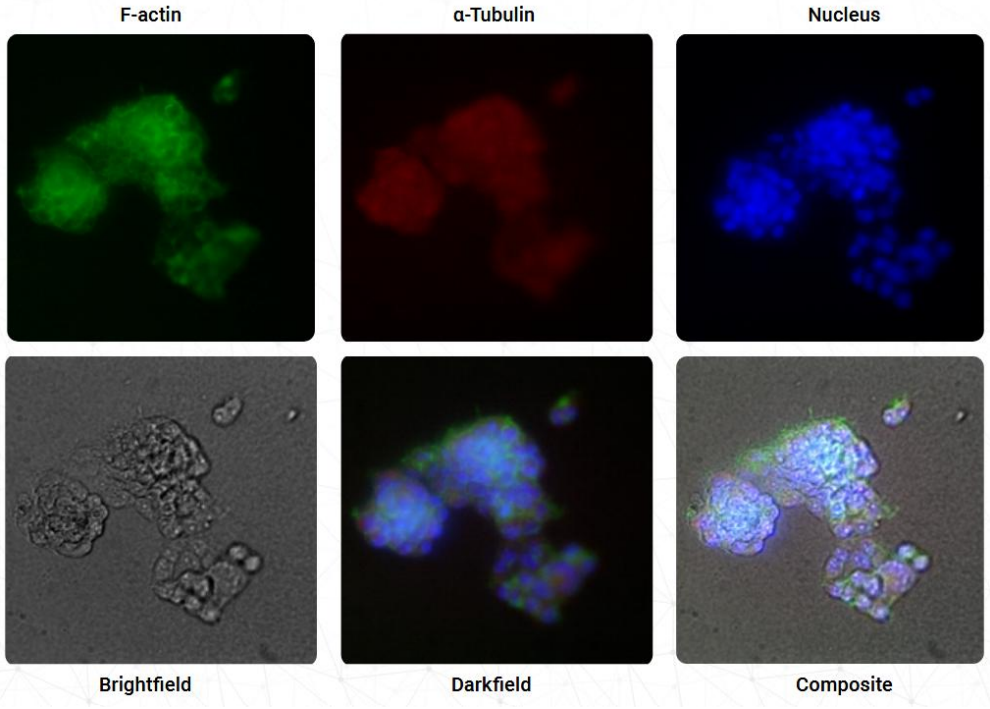

Fig. 2. HEK293 cells immunostaining on LifeGel. Objective 10x, optovar lens 2,5x.