Application Note
AN-F227-XXII-01

Detection and Quantification of Stem Cell Markers Using NYONE® Scientific

Willms A[1], Schaefer W[1], Philipp LM[2], Sebens S[2], Christmann T[1], Werdelmann B[1], Geisen R[1] & Pirsch M[1]
[1] SYNENTEC GmbH, Elmshorn, Germany
[2] Institute for Experimental Cancer Research, CAU + UKSH Kiel, Germany

Stem cells are characterized by longevity, continuous self-renewal, and the unique potential to develop into nearly any specialized cell type of the organism. Studying the properties of stem cells can help to increase the understanding of how diseases occur and enables the development of promising therapies in fields like regenerative medicine or oncology [1], [2]. One challenge is the identification of stem cells. Often, this is achieved by analyzing the expression of specific stem cell markers.

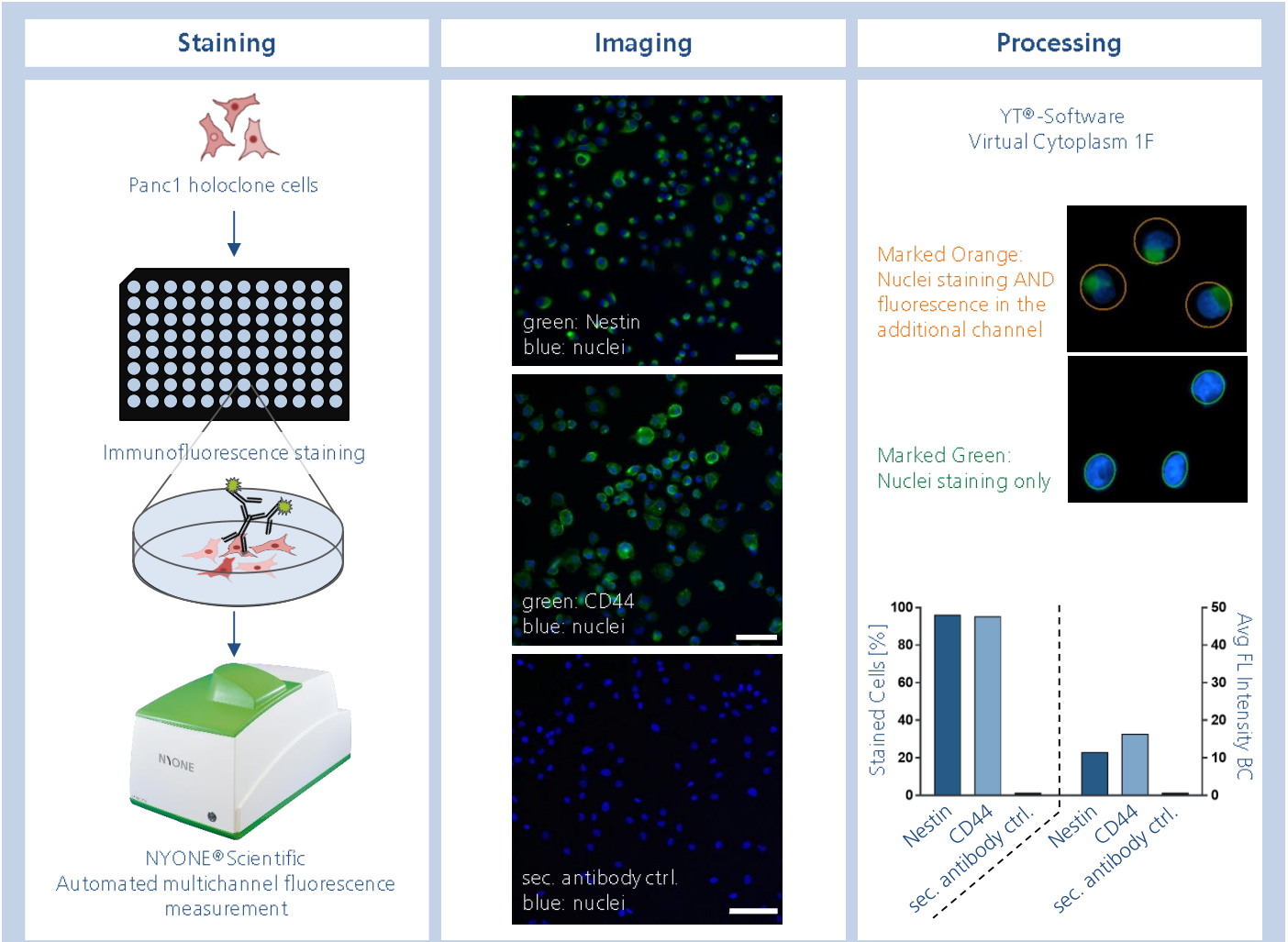

FIG. 1. METHOD OVERVIEW TO DETECT AND QUANTIFY STEM CELL MARKER EXPRESSION BY IMMUNOFLUORESCENCE
Panc1 holoclone cells (5,000 cells/well) were seeded in a 96 well microplate, fixed and stained using mouse-anti-Nestin or mouse-anti-CD44 antibody and anti-mouse Alexa Fluor 488 secondary antibody. Nuclei were visualized by Hoechst staining. Stainings were measured by SYNENTEC's imager NYONE® Scientific at 20x magnification. Results were evaluated with the image analysis application Virtual Cytoplasm 1F of YT®-Software. CD44, Cluster of Differentiation 44; sec. antibody ctrl., secondary antibody control; Avg FL Intensity BC, Average Fluorescence Intensity Background Corrected. (Scale bar: 100 µm)

Here we briefly describe an easy and fast method to detect stem cell marker expression and to quantify stem cells by immunofluorescence staining using SYNENTEC's automated imager NYONE® Scientific and its powerful YT®-Software (figure 1).
As a model to establish an immunofluorescence staining for stem cell markers, we used holoclone cells derived from the pancreatic cancer cell line Panc1 by single cell cloning as previously described [3]. These cells form morphologically characteristic colonies composed of small, densely packed, homogeneous cells with a clear and regular boundary, which are features of stem cell-like cancer cells (figure 2). In a 96 well plate format, we screened Panc1 holoclone cells for different proteins identified as markers of cancer stem cells by performing a conventional protocol for indirect immunofluorescence staining. Multichannel fluorescence measurements in ready-for-publication quality were done at 20x magnification using our automated imager NYONE® Scientific. The *Percentage of Stained Cells* and the *Average Fluorescence Intensity* are shown as two examples out of several results evaluated with the image analysis application Virtual Cytoplasm 1F of YT®-Software (figure 1).

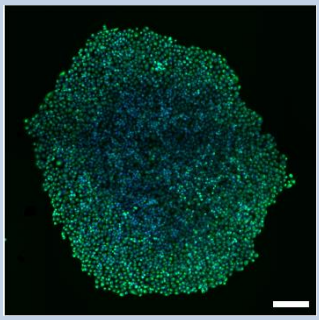

FIG. 2. PANC1 HOLOCLONE
Immunofluorescence staining of Panc1 holoclone colony. Green: Nestin, blue: nuclei. (Scale bar: 200 µm)

Summing up, here we present an easy method speeding up the work of conventional immunofluorescence stainings and imaging to identify cells with a stem cell-like phenotype. This application can be transferred to any other cell type and marker screening and can be extended with multiple stainings, enabling the characterization and quantification of cells in a high-throughput manner.

Material

Mouse anti-CD44 (156-3C11) antibody, Cell Signaling, Cat. No.: 3570
Mouse anti-Nestin (10C2) antibody, Cell Signaling, Cat. No.: 33475
Hoechst 33342, Thermo Fisher Scientific, Cat. No.: H1399
Goat anti-mouse Alexa Fluor 488, Thermo Fisher Scientific, Cat. No.: A-11001
96 well glass bottom microplate, greiner, Cat. No.: 655891
Poly-D-Lysine, gibco, Cat. No.: A38904-01
Bovine Serum Albumin Fraction V, Biomol Cat. No.: 1.400.100
Triton X-100, Merck, Cat. No.: 108603

References

[1] S. Yamanaka, "Pluripotent Stem Cell-Based Cell Therapy – Promise and Challenges," Cell Stem Cell, vol. 27, no. 4, pp. 523-531, Oct. 2020, doi: 10.1016/j.stem.2020.09.014.

[2] M. De Luca, A. Aiuti, G. Cossu, M. Parmar, G. Pellegrini, PG. Robey, "Advances in stem cell research and therapeutic development," Nat. Cell Biol., vol. 21, pp. 801-811, Jul. 2019, doi: 10.1038/s41556-019-0344-z.

[3] H. Knaack, L. Lenk, L.M. Philipp, L. Miarka, S. Rahn, F. Viol, C. Hauser, J.H. Egberts, J.P. Gundlach, O. Will, S. Tiwari, W. Mikulits, U. Schumacher, J.G. Hengstler, S. Sebens, "Liver metastasis of pancreatic cancer: the hepatic microenvironment impacts differentiation and self-renewal capacity of pancreatic ductal epithelial cells," Oncotarget, vol. 9, no. 60, pp. 31771–31786, Aug. 2018, doi: 10.18632/oncotarget.25884.

Acknowledgement

We thank the Institute for Experimental Cancer Research, especially Prof. Susanne Sebens, for outstanding support, fruitful discussions and a great working atmosphere during this cooperation.

SYNENTEC GmbH
Otto-Hahn-Str. 9A
25337 Elmshorn/Germany
Phone. +49 (0) 4121 46311-0
Email. service@synentec.com