New EdU Cell Proliferation Assays

S. Croce [1], S. Serdjukow [1], B. Oberleitner [1], T. Carell [2], T. Frischmuth [1]

[1] baseclick GmbH, Neuried 82061 Germany [2] Ludwig Maximiliam University, Munich 81377 Germany

This project has received funding from the European Union's Horizon 2020 framework program for research and innovation under grant agreement No 642023.

Introduction

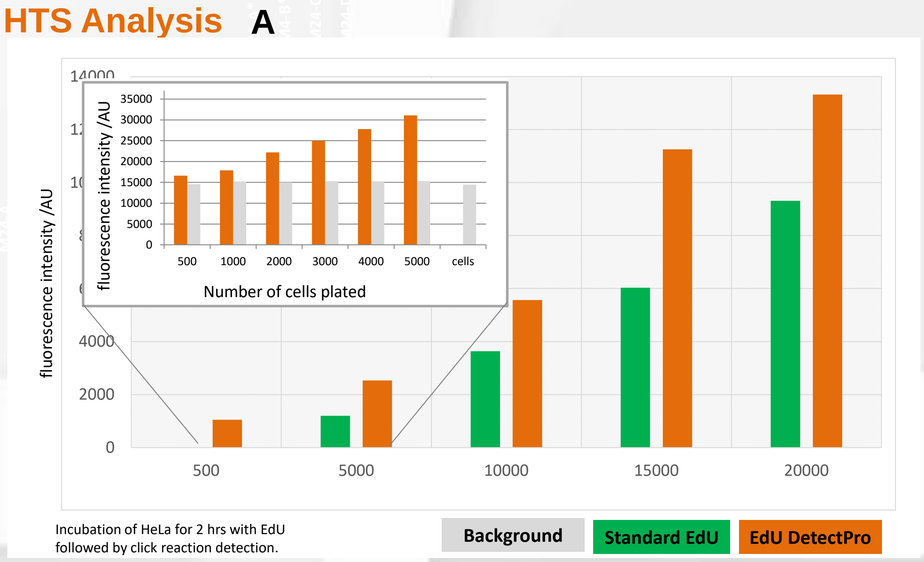
The detection of cell proliferation is of utmost importance for assessing cell health, determining genotoxicity or evaluating anticancer drugs. This is normally performed by adding nucleoside analogues like [^{3}H]thymidine or 5-bromo-2'-deoxyuridine (BrdU) to cells during replication, and their incorporation into DNA is detected or visualized by autoradiography or with an anti-BrdU-antibody respectively. Both methods exhibit several limitations. Baseclick EdU kits make use of an alkyne-modified thymidine analog, EdU, which gets phosphorylated and incorporated into DNA during DNA replication. The modified DNA can be detected by fluorescence microscopy, fluorescence activated cell sorting (FACS) or using plate-readers (HTS) after click reaction with a suitable dye azide.

Figure 1: The cell proliferation assay is based on the incorporation of the 5-ethynyl-2'-deoxyuridine (EdU), an analog to thymidine, into DNA during active cell cycle. For detection fluorescent dyes are clicked to the modified DNA.

EdU Detect Pro

Recently we developed a new generation of the EdU cell proliferation kit (EdU Detect Pro), reducing the background and therefore enhancing the sensitivity of the assay. (See HTS analysis Fig.2A, Microscopy images Fig.2B and FACS analysis Fig.2C).

HTS Analysis A



Microscopy B

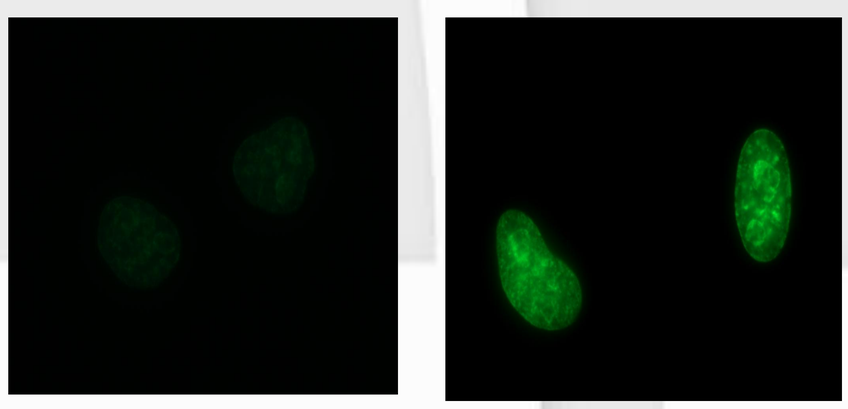


Figure 2: A) HTS analysis (fluorescence plate reader) and B) microscopic images of HeLa cells after EdU feeding and click detection.
Click detection of cell proliferation was done using an Alexa Fluor 488 analog, analysis of the samples was done using a fluorescence plate reader (A) or with a microscope (B). The standard EdU kit (left) and the enhanced EdU Detect Pro kit are compared; exposure time = 15 ms for microscopy. Fluorescence intensities for the HTS analysis (A) are background subtracted.

FACS Analysis C

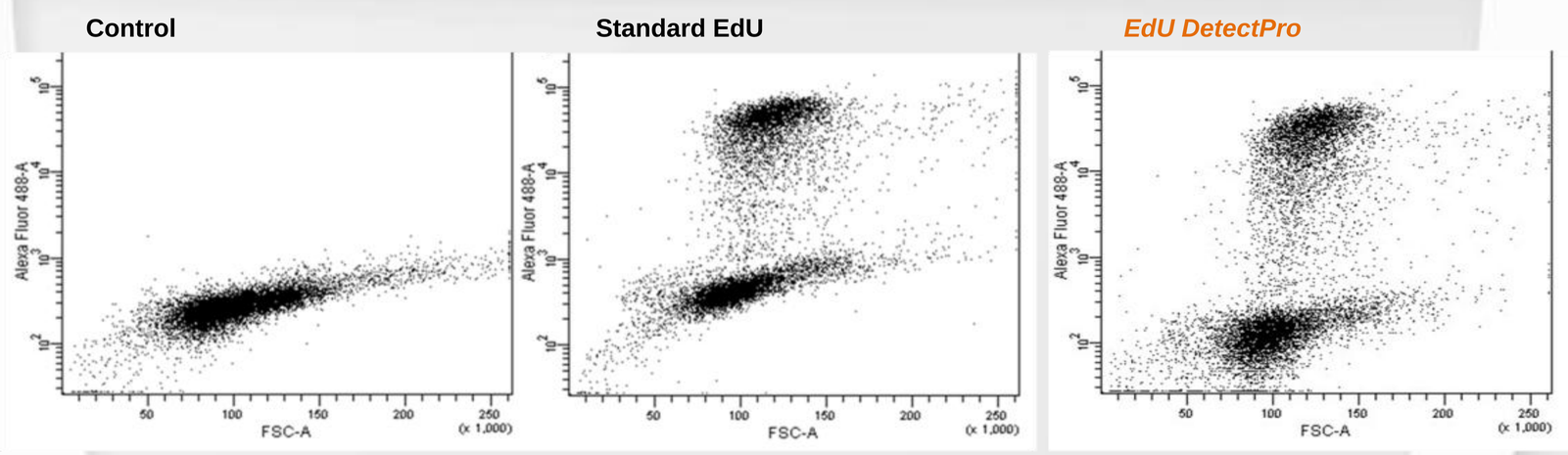


Figure 3: FACS analysis of EdU-fed Molm13 cells which were clicked with an Alexa Fluor 488 analog. The standard EdU labeling kit and the enhanced EdU Detect Pro kit were used for comparison; Alexa Fluor 488 voltage setting was adjusted according to the fluorescence signal of the control population (no EdU).

EdU Colorimetric Assay

We are currently working on a cell proliferation assay based on a colorimetric reaction. After the EdU is internalized into the DNA a click reaction covalently attaches a biotin-azide to the terminal alkyne groups. Next, the streptavidin-alkaline phosphatase conjugate is added to the samples and binds the biotin group. Addiction of an alkaline phosphatase substrate results in a detectable color change (see fig.C)

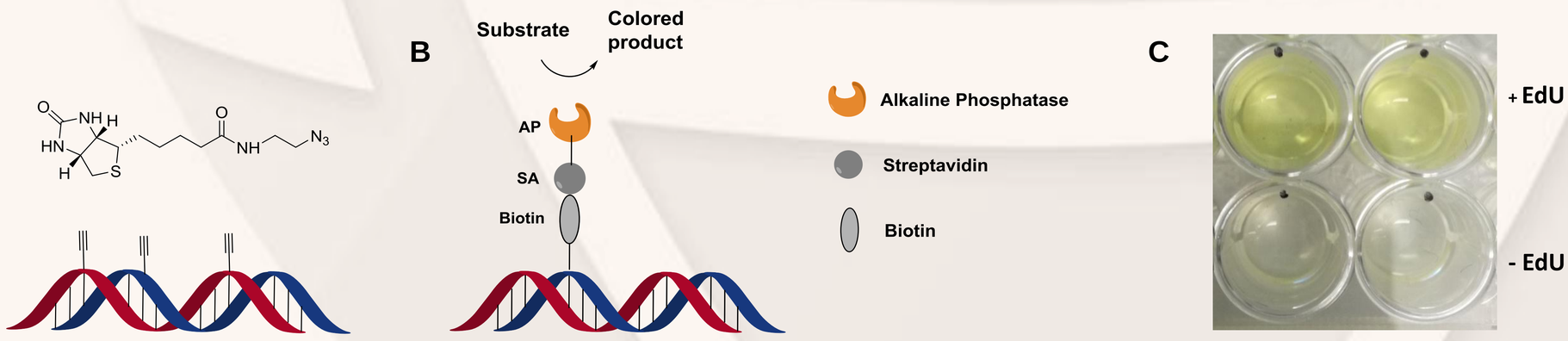


Figure 4: Colorimetric EdU assay principle and first results (C). A) A biotin azide is clicked to EdU modified DNA. B) The streptavidin of the streptavidin-alkaline phosphatase conjugate is binding to the clicked biotin, the alkaline phosphatase converts a non-colored substrate into a colored product. C) Colorimetric assay for cells with and without EdU incubation.

Conclusion

A new and significantly enhanced click-chemistry based EdU "DetectPro"-Kit for the analysis of cell proliferation has been developed. The new **DetectPro Kits** can be used for analysis by microscopy, FACS and by fluorescence plate readers (HTS). Currently a colorimetric EdU cell proliferation assay is being developed and has reached the proof-of-concept stage.

References

Salic A.; Mitchison T.; *Proceedings of the National Academy of Sciences* **2008** 105 **,**2415–2420.