Enhanced assay sensitivity with HCA ImagAmp™: An enabling signal amplification high content technology for technically challenging targets.

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Abstract

We utilized the HCA ImagAmp™ reagent kit in: (1) two typical high content analysis (HCA) assays for cytotoxicity, and in (2) a specific epigenetic assay monitoring the inhibition of dimethylated lysine 9 residue on histone H3 (H3K9me2). Signal amplification with HCA ImagAmp is achieved through enzyme-mediated deposition of multiple fluorophores in close proximity to a given antigen. The technology utilizes the ability of horseradish peroxidase (HRP) to convert fluorophore-labeled tyramide into a highly reactive molecule that will covalently bind to tyrosine residues in close proximity to the enzyme. For cytotoxicity assays, Cytochrome C release from mitochondria, as a marker for apoptosis, and phosphorylation of Histone H2A.X, as an indicator of DNA damage, were used to evaluate the performance of HCA ImagAmp. Amongst other results, exposure of HeLa cells to 0.5 µg/ml of the primary antibody was not sufficient to detect a specific signal from the secondary antibody (HCA ImagAmp™ kit, 350 ms). Apoptosis was induced by treatment of HeLa cells with 0.025 µg/ml of the primary antibody. With HCA ImagAmp, 73.5% of the cells were found to be apoptotic, compared to only 2.8% in untreated controls. In contrast, Alexa Fluor®-labeled secAb stained cells, when imaged under exactly the same conditions, did not used a significant staining.

1. Assay Principle

2. DNA Damage

DNA damage is induced by Neocarzinostatin (NCS), which causes DNA double stranded breaks. As a result, phosphorylation of Histone H3 K occurs, which can be detected with an antibody against the phosphorylated protein (Regaux et al., 1990). After staining with HCA ImagAmp, NCS treatment resulted 98.4% of the DNA damage, in contrast to 1.5% without treatment. Using Cytochrome C labeling, the increase in the concentration of the primary Ab required and the exposure time.

3. Cytochrome C

Cytochrome C is a mitochondrial protein; during apoptosis it loses its specific localization and becomes more evenly distributed throughout the cell cytoplasm (Douglas-Henry et al., 1996). Apoptosis was induced by treatment of HeLa cells with 0.025 µg/ml of the primary antibody. With HCA ImagAmp, 73.5% of the cells were found to be apoptotic, compared to only 2.8% in untreated controls. In contrast, Alexa Fluor®-labeled secAb stained cells, when imaged under exactly the same conditions, did not used a significant staining.

4. Histone H3 Epigenetic Modifications

UNC0638 is a histone methyltransferases inhibitor. When the cells are treated with this compound, a lower level of H3K9me2 is observed (Regaux et al., 1996). In comparison to 3A6-Neo Fluor 488 staining, HCA ImagAmp 488 strongly increased the H3K9me2 specific signal in untreated conditions. The ratio of the signal to background ratio for HCA ImagAmp labeled cells increased significantly, thereby improving the ability to detect changes in H3K9me2 levels.

5. Materials

6. Methods

To perform immunofluorescence staining a standard protocol was used. Briefly, HeLa cells were seeded at a density of 6000 per well in 96-well plates, fixed for 15 min with 4% paraformaldehyde in CellStainer-™ ELX plates. After fixation, the cells were permeabilized with 0.5% Triton X-100 and blocked by incubation with Blocking Reagent® for 1 h. The cells were then incubated with the corresponding antibody in a 1:100 dilution for 1 h at room temperature. After three washes, the cells were stained with Cryptate®-labeled secAb stained cells, when imaged under exactly the same conditions, did not used a significant staining.

7. References

8. Summary

The increase in sensitivity is highlighted by the fact that HCA ImagAmp, in comparison to conventional confocal microscopy, could detect significantly less DNA damage for significant reduction of the concentration of primary Ab needed and requires much shorter exposure times.

In addition, the NCS-titration experiments show that the amplified signal enables specific detection of signal intensity at low levels of DNA damage, allowing for limit towards lower concentrations of NCS.

Finally, for technically challenging targets such as the detection of few abundance epigenetic histone methyl marks, the HCA ImagAmp™ reagent kit clearly represents a markedly better detection method than conventional IF.