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Homogeneous Antibody-Binding Assay Using SYNENTEC's High-Throughput Microscope NYONE® Scientific

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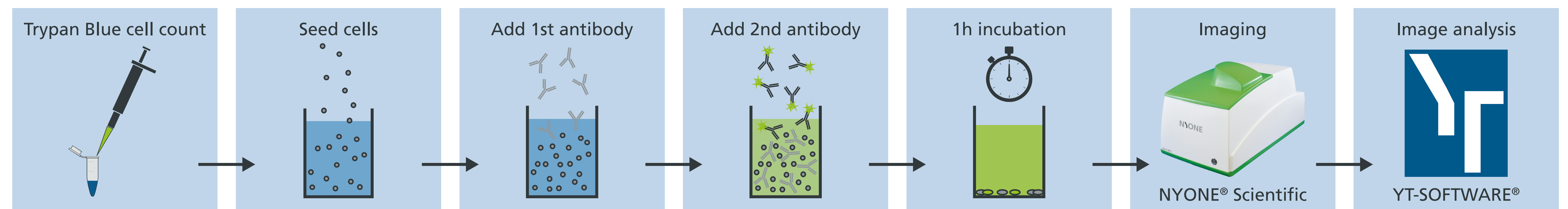
Introduction

The production of high-affinity, antigen-specific antibodies is crucial for various purposes in research, diagnosis, and therapy. Antibody-producing cells are commonly generated using hybridoma technology and single cell cloning (SCC). However, the selection of high-producing clones is challenging and requires a reliable method. The assay should need minimal amounts of antibody sample and no wash steps. Therefore, we aimed to develop a simple homogeneous antibody binding assay using our automated microscope NYONE® Scientific. As a reference model, we used an anti-HER2 antibody with the HER2-expressing breast cancer cell line SK-BR-3 as target cells and HER2-negative MDA-MB-468 cells as control. Cells were seeded into 384-well plates, followed by primary anti-HER2 antibody and fluorescence-labeled secondary antibody. The entire 384-well plate was imaged in less than 6 minutes, and the images were analyzed using the Suspension Cell AB Binding (1F) application of YT-SOFTWARE®.

Benefits

- Screening without plate washing
- Increased reproducibility and accuracy through simple assay design
- Fast imaging for high-throughput screening
- Detection of weak signal with high sensitivity up to 1 ng/mL
- Same device and software as for single cell cloning

Method

Trypan Blue cell count → Seed cells → Add 1st antibody → Add 2nd antibody → 1h incubation → Imaging (NYONE® Scientific) → Image analysis (YT-SOFTWARE®)

Results

control | low concentration | optimal binding | high concentration | Hook effect

primary antibody
fluorophore conjugated secondary antibody
antigen
cell

brightfield channel / fluorescence channel / Image processing

Suspension Cell AB Binding (1F)

detection of the cells in the brightfield channel

determination of fluorescence intensity within the cells (background corrected)

labeling of fluorescence positive cells

100 µm

Avg Fluo CH1 Intensity BC_{norm} [-] vs 1st antibody [ng/mL]

- ■ SK-BR-3
- ▼ MDA-MB-468