SARTORIUS

Application Note

July, 2023

Keywords or phrases:
Research Use Only (RUO), Cytokines, Growth Factors, Neuronal Differentiation, Live-Cell Analysis, Live-Cell Immunocytochemistry (ICC), Neurite Outgrowth, Neurotrack

Optimization of SH-SY5Y Differentiation Using Growth Factors and Cytokines

Jasmine Trigg, Daryl Cole, Natasha Lewis, Nicola Bevan, Tim Dale
Sartorius UK Ltd, Units 2 & 3 The Quadrant, Newark Close, Royston, Hertfordshire, SG8 5HL, UK

Correspondence
E-Mail: AskAScientist@sartorius.com

Introduction

For neuroscientific research a crucial component is utilizing appropriate model systems which are translational for studying human development and disease. The use of *in vitro* models has greatly enhanced the field and traditionally these include primary rodent neuronal cultures, end-point slice cultures, and immortalized cell lines.[1] Growth factors and cytokines are signaling molecules that play critical roles in these models and are involved in a wide range of functions including cell growth, differentiation, and survival.[2,3]

SH-SY5Y cells, which exist as two morphologically distinct phenotypes including epithelial-like (S-type) or neuroblast-like (N-type), are a subclone of the human SK-N-H parental neuroblastoma cell line.[4,5] They are a widely used *in vitro* model as they can be differentiated into a neuronal-like phenotype. Additionally, unlike primary rodent or induced pluripotent stem cell (iPSC)-derived neurons, they afford the benefit of being open to low-cost large-scale expansion, amenable to genetic modification, and express human-specific proteins.[6,7] SH-SY5Y cells can be differentiated through several different mechanisms depending on the phenotype of interest including all-trans retinoic acid (atRA)[8], phorbol esters[9], and neurotrophic factors such as insulin-like growth factor 1 (IGF-1)[10], brain-derived neurotrophic factor (BDNF)[11], and bone morphogenic protein 4 (BMP-4)[12]. Typically, upon differentiation into a neuronal-like phenotype, these cells cease proliferating, develop neurite-like extensions, form functional synapses, and express mature neuronal markers such as microtubule associated protein (MAP) and neural cell adhesion molecule (NCAM or CD56).[11,13]

Currently, these protocols are not well-defined and can induce variable and heterogenous populations of neurons.[1] Moreover, standard approaches to studying neuronal differentiation rely on end-point assays including high-content analysis, fixed immunocytochemistry (ICC), or flow cytometry. These often require destructive protocols preventing visual assessment of morphological changes and provide limited kinetic information. To successfully use these *in vitro* models across a variety of neuroscientific research areas, approaches to reproducibly generate homogeneous neuronal cultures and technology pipelines to visualize and temporally quantify these complex models are required.

In this application note, we demonstrate robust differentiation of human SH-SY5Y cells into neuronal-like cells using Sartorius Research Use Only (RUO) Growth Factors and Cytokines and the utility of Incucyte® Live-Cell Imaging and Analysis to kinetically visualize and quantify changes in proliferation, morphology, and cell surface antigen expression.

Assay Principle

Sartorius offers a range of high-quality human RUO Growth Factors and Cytokines that are produced using recombinant DNA technology and do not contain any animal-derived components. These growth factors are of high-purity, low endotoxicity, and can be used for the long-term differentiation or maintenance of neuronal cultures in a non-perturbing and reproducible manner.

The Incucyte® Live-Cell Analysis System enables kinetic monitoring and quantification of cell cultures using HD Phase-contrast and fluorescence images, which are automatically quantified via integrated software. Purpose-built Incucyte® Neurotrack Analysis Software enables the quantification of neuronal outgrowth to be measured in mono-cultures label-free without perturbing sensitive cultures. Additional insights can be gained using Incucyte® Fabfluor Antibody Labeling Dyes, which, once complexed to Fc containing antibodies of interest, enable dynamic monitoring of surface antigen expression in live cells. Importantly, these combined approaches enable multiparametric analyses of cell morphology and phenotype and the temporal association of these with neuronal differentiation.

Materials and Methods

SH-SY5Y human neuroblastoma cells were stably transfected with Incucyte® Nuclight Orange Lentivirus, a nuclear restricted fluorescent marker. Undifferentiated SH-SY5Y cells were cultured in DMEM/F12K (1:1) supplemented with 10% fetal bovine serum (FBS) and 1% Pen/Strep (complete media).

For differentiation, SH-SY5Y cells were seeded into 96-well TPP plates (Cat. No. 92096) and allowed to adhere until a confluency of ~15% was achieved. As outlined in Figure 1, we used two methods for SH-SY5Y differentiation into neuronal-like phenotypes. These utilized atRA (10 µM) and/or Sartorius RUO Recombinant Human Growth Factors or Cytokines including BMP-4 (Cat. No. CYK-0025-0006) and BDNF (Cat. No. CYK-0100-0008). Sartorius RUO Glia-Derived Neurotrophic Factor (GDNF; Cat. No. CYK-0025-0009) and Neurotrophin-3 (NT-3; CYK-0025-0020) were also used in optimization studies. Cultures were monitored in an Incucyte® Live-Cell Analysis System at 2 – 4 hour intervals for up to 14 days. Media refreshes were performed every 2 – 3 days.

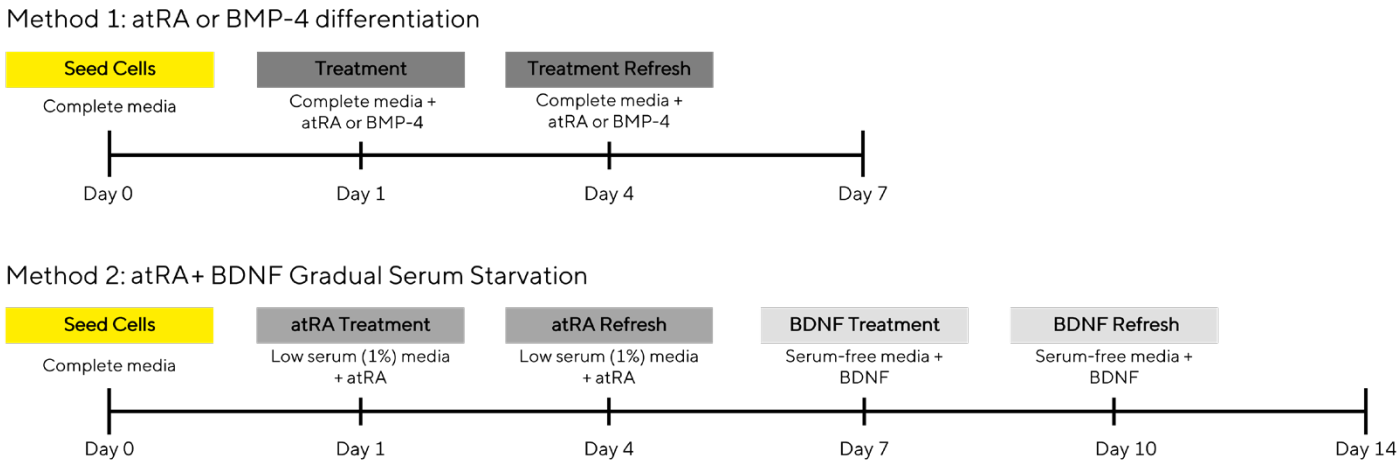

Figure 1. Schematic of methods for neuronal differentiation. Methods outlined for differentiating human SH-SY5Y neuroblastoma cells into neuronal-like phenotypes. These utilize Sartorius RUO Growth Factors and Cytokines and the Incucyte® Live-Cell Analysis System to enable image-based measurements of differentiation in real-time.

The Incucyte® Live-Cell immunocytochemistry (ICC) assay involves a simple mix-and-read, no wash protocol in a 96/384-well format as demonstrated in Figure 2. Once labeled with isotype matched Incucyte® IgG1 Fabfluor-488 (Cat. No. 4745) or IgG2a Fabfluor-488 (Cat. No. 4743) Labeling Dyes, the Fabfluor-antibody complex in combination with Incucyte® Opti-Green background suppressor was added to live-cells. Azide-free antibodies of interest were used to minimize perturbance to long-term sensitive cultures.

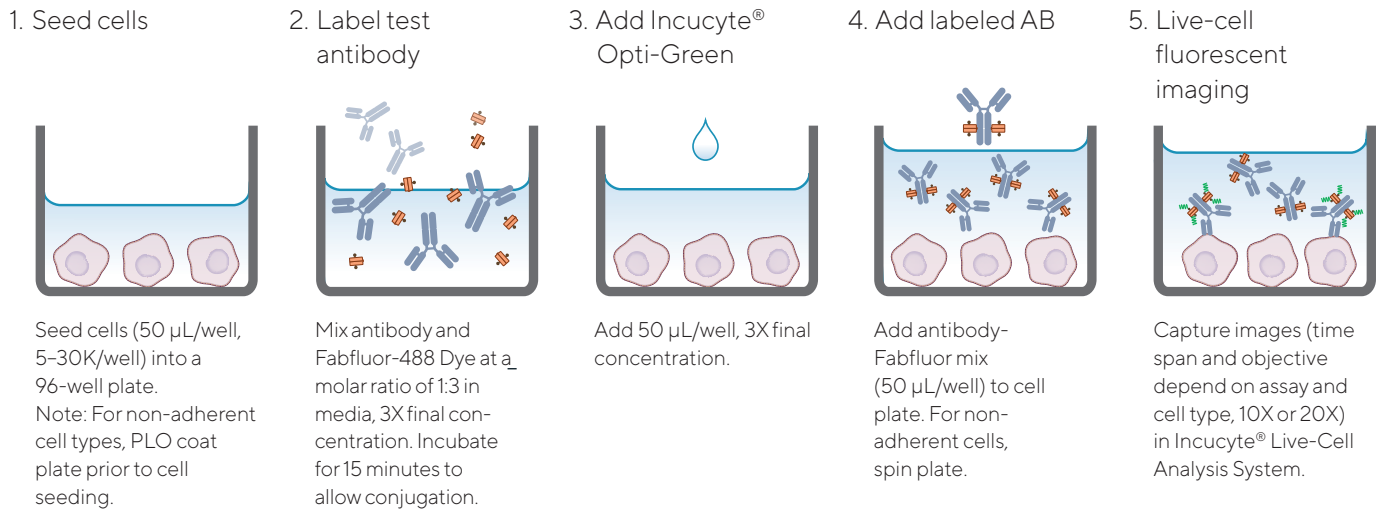

Figure 2. Quick guide of Incucyte® Live-Cell Immunocytochemistry (ICC) protocol. The simple mix-and-read protocol utilizes Incucyte® Fabfluor Antibody Labeling Dyes and the Incucyte® Live-Cell Analysis System for image-based fluorescent measurements of surface antigen expression.

Quantification of Dynamic Changes During Neuronal Differentiation

The Incucyte® Neurotrack Analysis Software Module automatically segments phase images and generates metrics describing neurite length, neurite branching, and cell-body clusters (count or area). Additional nuclear count measurements can be obtained when cells contain a fluorescent nuclear label. Here we demonstrate how this software can be used to quantify cell proliferation and morphological changes, such as neuronal outgrowth, as indicators of neuronal differentiation (Figure 3). SH-SY5Y cells stably expressing Incucyte® Nuclight Orange were differentiated using a gradual serum-starvation method with the sequential introduction of atRA (10 µM) and BDNF (50 ng/mL) (method 1). Representative phase and fluorescence images (Figure 3A) for differentiated SH-SY5Y cells reveal a mature neuronal-like phenotype with reduced cell body size and extended neurites on day 14. The segmentation masking of fluorescent cell nuclei or label-free segmentation of neurites and cell-body clusters are also shown. The resulting quantification revealed that proliferation is inhibited over-time for differentiated compared to undifferentiated cells (Figure 3B) and we observed a kinetic increase in neurite length following BDNF addition compared to serum-free control (Figure 3C).

The Incucyte® Live-Cell ICC Assay permits dynamic quantification of cell surface antigen expression. Automated imaging and integrated analysis tools enable image-based measurements of fluorescence over the entire assay time course and, alongside Opti-Green, minimize background fluorescence. In the absence of expressed specific antigen, little-to-no signal is observed on the cells. Cell surface expression can be quantified using several metrics, including fluorescence area (Total Green Object Area), fluorescence intensity integrated over the area of detectable fluorescence (Total Green Object Integrated Intensity), or through normalizing the fluorescence area to cell coverage (Green Object Confluence/Phase Confluence).

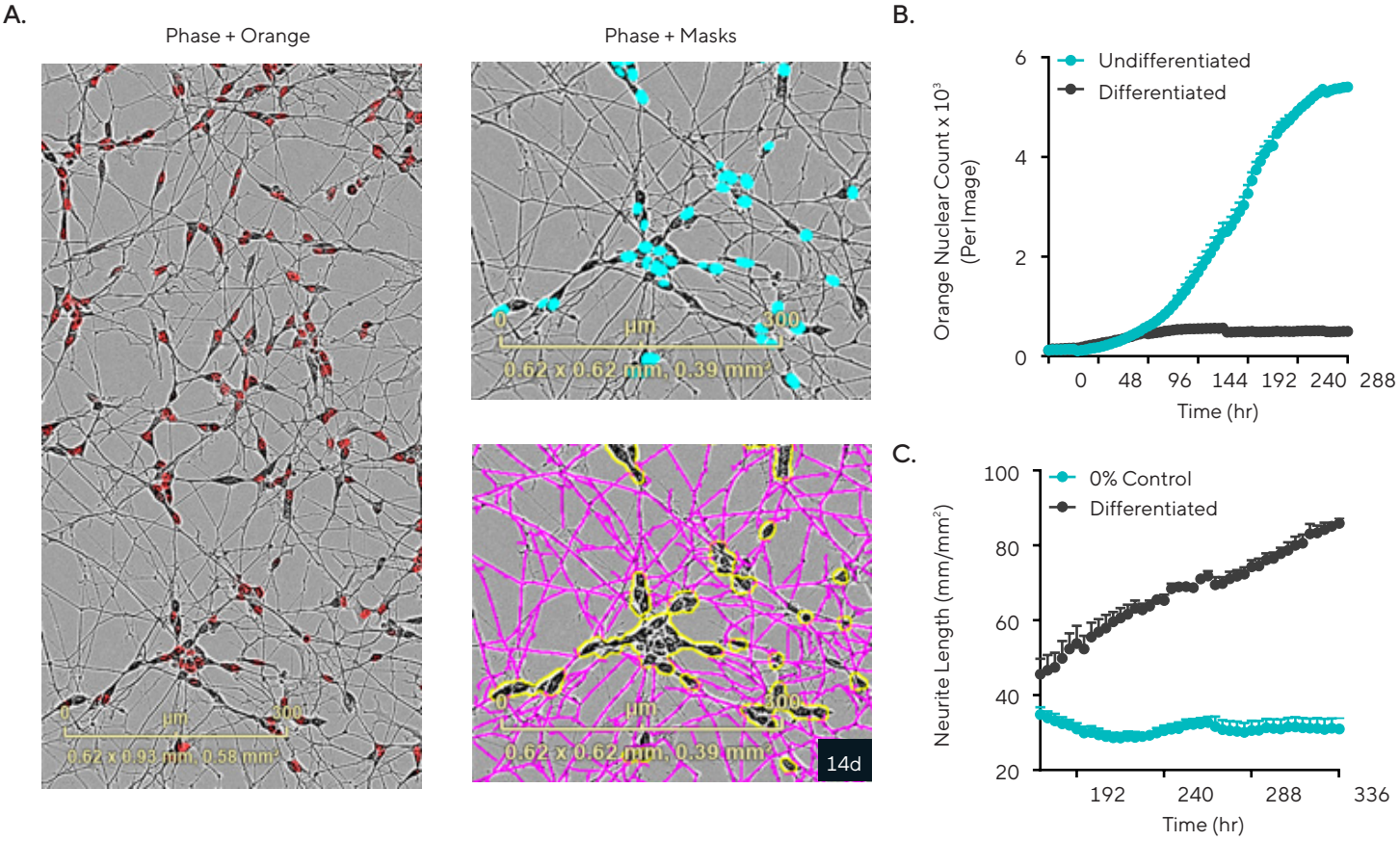

Figure 3. Visualization and quantification of neuronal differentiation. SH-SY5Y-Nuclight Orange cells were differentiated by gradual serum starvation (10 – 0%) in combination with atRA (10 µM) and Sartorius Recombinant Human BDNF (50 ng/mL). Images were acquired at 10X over 14 days using the Incucyte® Live-Cell Analysis System and quantified using integrated Incucyte® Neurotrack Analysis Software. A) Representative phase and fluorescence images and segmentation masks of fluorescence cell nuclei (blue), label-free neurites (pink) and cell-body clusters (yellow outline) shown at 14 days. B) Time courses display change in orange nuclear count and neurite length. Data presented as mean ± SEM, n = 3 replicates.

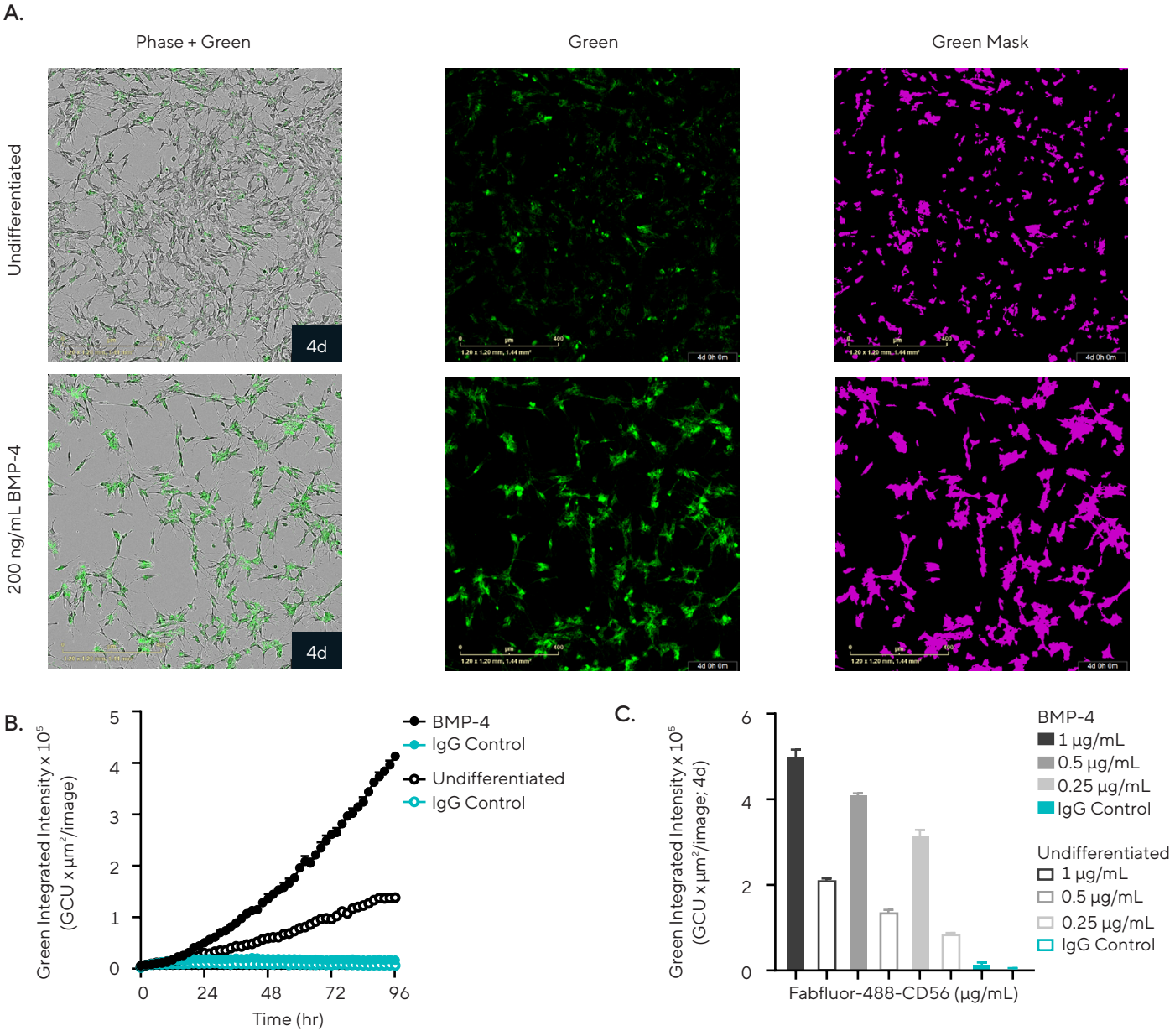

Figure 4. Live-cell immunochemistry (ICC) labeling optimization using BMP-4 model of differentiation. SH-SY5Y-Nuclight Orange cells were treated with a concentration range of Fabfluor-488-CD56 antibody complexes (0.25 – 1 µg/mL) in the presence of Opti-Green under undifferentiated or BMP-4 (200 ng/mL) differentiated conditions. A) Representative phase and fluorescence images for undifferentiated and differentiated cells on day 4 with Fabfluor-488-CD56 shown in green and segmentation mask in magenta. B) Time course for Total Green Integrated Intensity over 4 days for 0.5 µg/mL Fabfluor-488-CD56 or IgG isotype control in undifferentiated or differentiated conditions. C) Total Green Integrated Intensity on day 4 shown for all conditions. Data presented as mean ± SEM, n = 3 replicates.

To exemplify quantification of changes in CD56 surface antigen expression during BMP-4 differentiation we optimized live-cell ICC labeling using a concentration range of Fabfluor-488-CD56 antibody complexes (Figure 4). Shown are representative images and the segmentation mask used (Figure 4A). Quantification of the green integrated intensity, which accounts for both changes in green intensity and cell area, showed that undifferentiated cells have a basal level of CD56 expression which kinetically increases with BMP-4 differentiation (Figure 4B). This increase in CD56 expression was antibody-complex concentration-dependent across both conditions, whilst little-to-no fluorescence was detected for IgG isotype controls (Figure 4C). To determine an optimal antibody-complex concentration, we assessed the assay windows between undifferentiated and differentiated conditions and directly confirmed that we could visualize and quantify fluorescence in both the cell body and neurite-like extensions. We selected 0.5 µg/mL for use in subsequent experiments as it provided a good assay window and the desired distribution of fluorescence. Overall, these methods can robustly be used to quantify the dynamic changes that occur with neuronal differentiation.

Growth Factor and Cytokine Concentration Assessment

To optimize concentrations of differentiation-inducing treatments, SH-SY5Y-Nuclight Orange cells were treated with a concentration range of four Sartorius Growth Factors and Cytokines including BDNF, BMP-4, GDNF, and NT-3, and monitored using the Incucyte® Live-Cell Analysis System (Figure 5). The microplate view reports orange nuclear count normalized to pre-treatment over 4 days (Figure 5A) and we observed no toxicity for all neurotrophic factors at the concentrations studied compared to positive control camptothecin (CMP). BMP-4 induced modest inhibition on cell proliferation across all concentrations compared to vehicle (Figure 5B). HD images also confirmed subtle morphological changes in BMP-4 treated cells with the development of neurite-like extensions, consistent with known differentiating effects.[12] The data demonstrates the ability to accurately monitor cell viability in a 96-well throughput and highlights the reproducibility of Sartorius RUO Growth Factors and Cytokines.

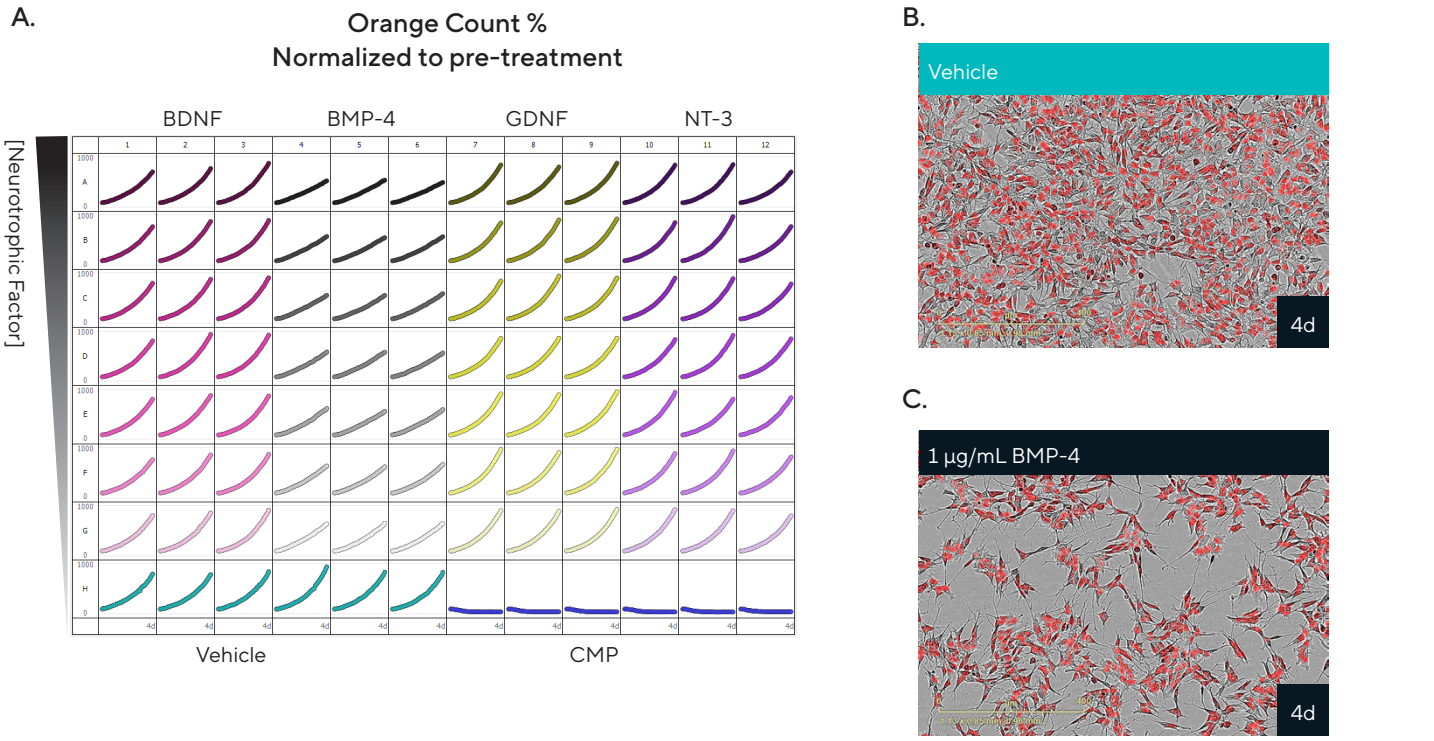

Figure 5. Neurotrophic factor concentration range assessment. SH-SY5Y Nuclight Orange cells were treated with neurotrophic factors BDNF, BMP-4, GDNF, and NT-3 (1 – 0.01 µg/mL) and monitored using the Incucyte® Live-Cell Analysis System. 10 µM Camptothecin (CMP) was used as a positive control for cell death. A) Microplate graph shows nuclear count normalized to pre-treatment over 4 days for all treatment conditions. B) Blended phase and orange images shown at 4 days for 1 µg/mL BMP-4 and vehicle (10X). Data shown as mean ± SEM, n = 3 - 6 replicates.

Monitoring Proliferation and Cell Surface Antigen Expression during BMP-4 differentiation

RA is a Vitamin A derivative commonly used to inhibit cell growth and rapidly differentiate SH-SY5Y cells from a N-type to a neuronal-like phenotype. However, studies suggest that RA does not strongly promote neuronal marker expression and long-term treatment results in heterogenous populations due to transdifferentiation occurring between N- and S-types.[14,11] To avoid these limitations, and obtain higher-quality neuronal cultures, different approaches are increasingly being applied. One approach is the use of BMP-4, a signaling molecule that is part of Transforming growth factor-beta (TGF-β) protein superfamily.[15] BMP-4 appears to play crucial roles in neural stem cell development, axonal growth, and has been shown to promote neuroblastoma differentiation.[16,17]

We previously established that BMP-4 induced morphological changes in SH-SY5Y cells consistent with a neuronal-like phenotype (Figure 5). Subsequently, we investigated any associated changes in surface antigen expression through kinetically examining CD24, a N-type neural precursor marker highly expressed in undifferentiated cells, and the neuronal marker CD56.[18,12] SH-SY5Y Nuclight Orange Cells were treated with atRA or a concentration range of BMP-4 in the presence of Incucyte® Fabfluor-488 Dye complexed to CD24, CD56, or isotype controls (Figure 6). Fluorescence images indicated changes in nuclear count, and expression and distribution of CD56 for BMP-4 compared to atRA and undifferentiated cells (Figure 6A). Quantification showed BMP-4 induced a concentration-dependent inhibitory effect on proliferation compared to control over 5 days with maximal inhibition being slightly lower than that observed for atRA (2.26 ± 0.3, 1.56 ± 0.1, 4.99 ± 0.4 x10^3 count per image, for BMP-4, atRA, or control, respectively) (Figure 6B). For CD24 we observed a marked decrease for atRA and a modest concentration-dependent decrease for BMP-4 suggesting partial loss of neural precursor expression (Figure 6C). In contrast, for CD56 we observed a slight increase in expression for atRA and an enhanced concentration-dependent increase for BMP-4 (EC_{50} value of 4.72 ng/mL, 95% CI [3.65, 6.20] (Figure 6D)). Overall, these data confirm that BMP-4 differentiation induces homogenous neuronal-like phenotypes and highlights how this approach facilitates insights into neuronal differentiation and cellular interconversion.

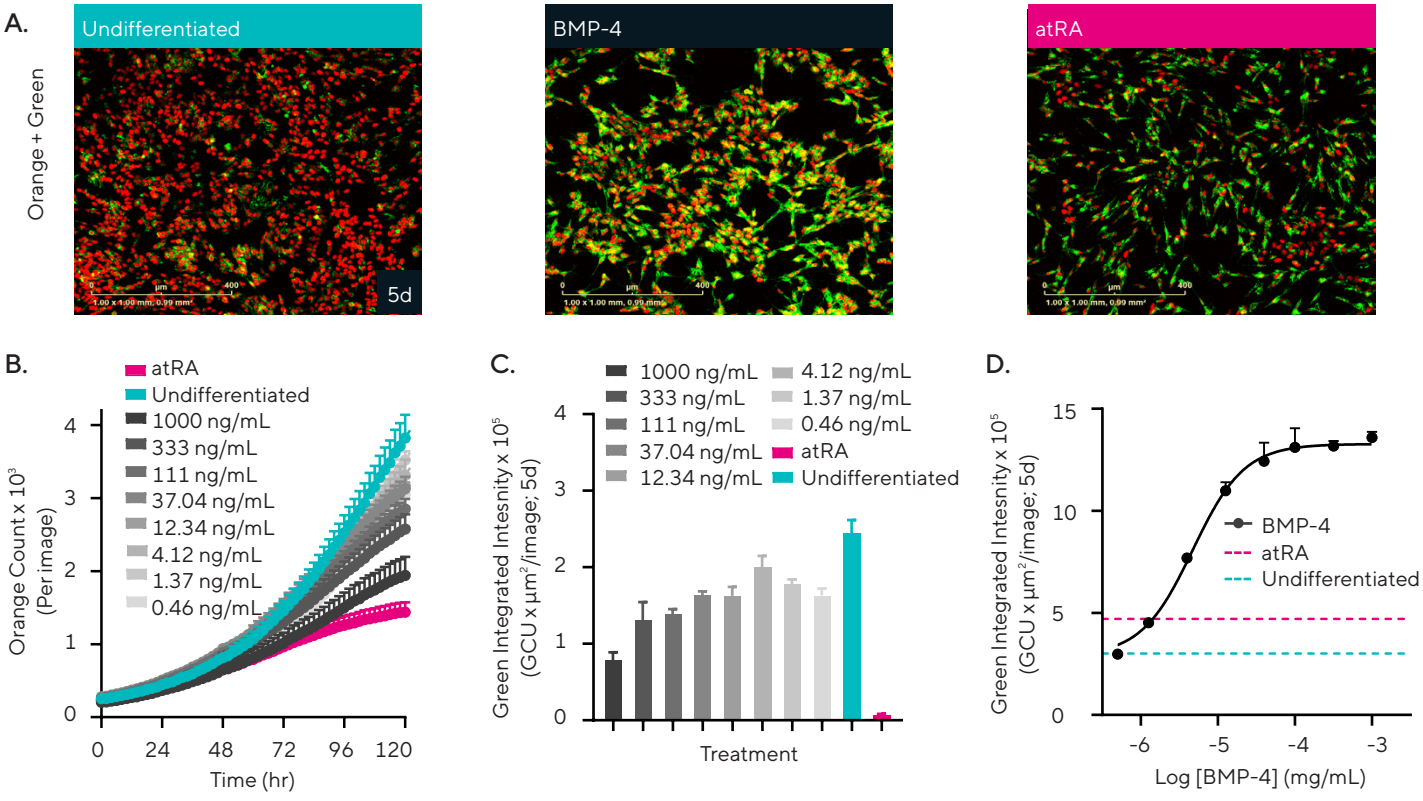

Figure 6. BMP-4 differentiation induces concentration-dependent changes in proliferation and surface antigen expression. SH-SY5Y Nuclight Orange cells were differentiated with BMP-4 (1000 – 0.41 ng/mL) or atRA (10 µM) and treated with either Incucyte® Fabfluor-488 antibody complexed to CD24, CD56, or IgG control. A) Representative Green and Orange blended fluorescence images shown for undifferentiated, BMP-4 (1000 ng/mL) or atRA differentiated cells at 5 days. B) Time course shown for orange nuclear count over 5 days. C) Total Green Integrated Intensity shown for CD56 at 5 days. D) Transformed data shows concentration response curve to BMP-4 for CD56 expression at 5 days; vehicle (teal) and atRA (magenta) plotted as dashed lines. Data presented as mean ± SEM, n = 3 replicates.

Kinetic Assessment of BDNF Differentiation

BDNF protein, a member of the neurotrophin family, plays essential roles in the regulation of neuronal development, including differentiation, axonal growth, synapse formation, maturation, and maintenance.[19,20] It is used across several *in vitro* applications, including the differentiation of neural progenitor cells, cortical organoids, and immortalized human cell lines.[21,22]

One established protocol for the differentiation of SH-SY5Y cells to a neuronal-like phenotype is based on gradual removal of serum from the media and sequential treatments with atRA and BDNF.[11] This method has been shown to augment the differentiating properties of atRA and generate homogeneous populations of cells with enhanced neuronal-like characteristics, including a more mature morphology, elevated neuronal marker expression, and improved cell survival.[11,23,24] To assess this method and examine the effects of BDNF, SH-SY5Y-Nuclight Orange cells were differentiated using a gradual serum-starvation method with the addition of atRA (10 µM) followed by a concentration-range of BDNF (method 2). Differentiation was performed in the presence of Incucyte® Fabfluor-488 Dye complexed to CD56, housekeeping marker CD71, or IgG isotype control.

Phase images reveal the temporal morphological changes occurring during differentiation using this method (Figure 7A). In undifferentiated form, SH-SY5Y neuroblast-like cells are non-polarized with few truncated processes; during the initial stages of atRA differentiation cells elongate and form neurite-like extensions. In later stages with BDNF, the cell bodies retract and form clusters with neurites developing extensive, branched networks similar to primary or iPSC-derived neurons. Green fluorescence images show the corresponding CD56 expression (Figure 7B). The results revealed temporal changes in neuronal-specific CD56 expression throughout the differentiation process (Figure 7C), with a rapid increase observed initially and more subtle changes in intensity and distribution observed at later time points. Little-to-no expression was observed for IgG control and CD71 showed stable and homogeneous expression throughout. Additionally. we observed concentration-dependent differences in CD56 expression, with a bell-shaped response to BDNF at 14 days (Figure 7D).

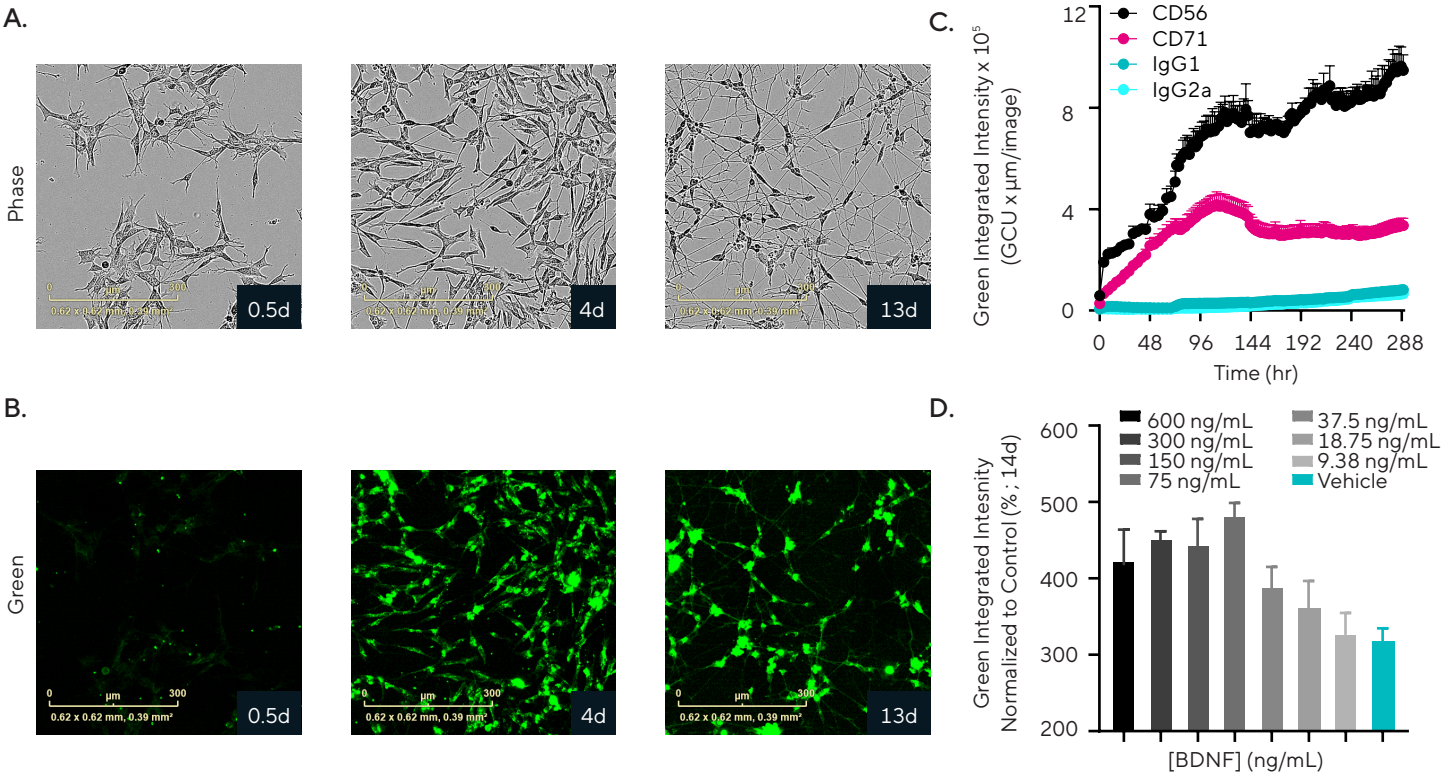

Figure 7. Kinetic monitoring of cell surface antigen expression during SH-SY5Y differentiation into a mature neuronal-like phenotype. SH-SY5Y Nuclight Orange cells were differentiated by gradual serum starvation (10 – 0%) in combination with atRA (10 µM) and a concentration-range of Sartorius Human Recombinant BDNF (600 – 9.38 ng/mL) for up to 14 days. Differentiation was performed in the presence of Incucyte® Fabfluor-488 antibody complexed to CD56 (neuronal marker), CD71 (housekeeping protein), or IgG control and Opti-Green. Timelapse Phase (A) and fluorescence images (B) shown for differentiation using atRA and 50 ng/mL BDNF. C) Time course of Green Integrated Intensity shown for all conditions. D) Bar graph shows CD56 expression in response to BDNF or vehicle control at 14 days. Green integrated intensity was normalized to IgG control to account for some observed autofluorescence of clustered cell bodies in mature cultures. Data presented as mean ± SEM, n = 3 - 5 replicates.

Additionally, we quantified morphological changes using Incucyte® Neurotrack Analysis Software. Shown are HD phase contrast images for differentiated SH-SY5Y cells and the label-free segmentation used to mask neurites and cell body clusters (Figure 8A). We observed a mature homogeneous neuronal-like phenotype with morphological differences detected at different concentrations of BDNF compared to vehicle (serum-free media). Transformation of the data showed BDNF induced a concentration-dependent effect on neurite development, including neurite length, branch points, and cell-body cluster area (Figure 8B). Notably, the absence of BDNF results in cellular apoptosis as visualized in the vehicle images. This is suggestive of differentiated SH-SY5Y survival being dependent on BDNF in serum-free conditions and consistent with BDNF being a known regulator of cell survival in neuronal cultures.[1,11]

Overall, these data confirm that this method reproducibly induces temporal changes in morphology and surface antigen expression consistent with neuronal differentiation, with the commonly used concentration of 50 ng/mL BDNF proving to be within an optimal range. The data also highlights the amenability of this multiplexed approach to optimize long-term differentiation protocols in a 96-well microplate throughput using high-quality RUO Growth Factors and Cytokines.

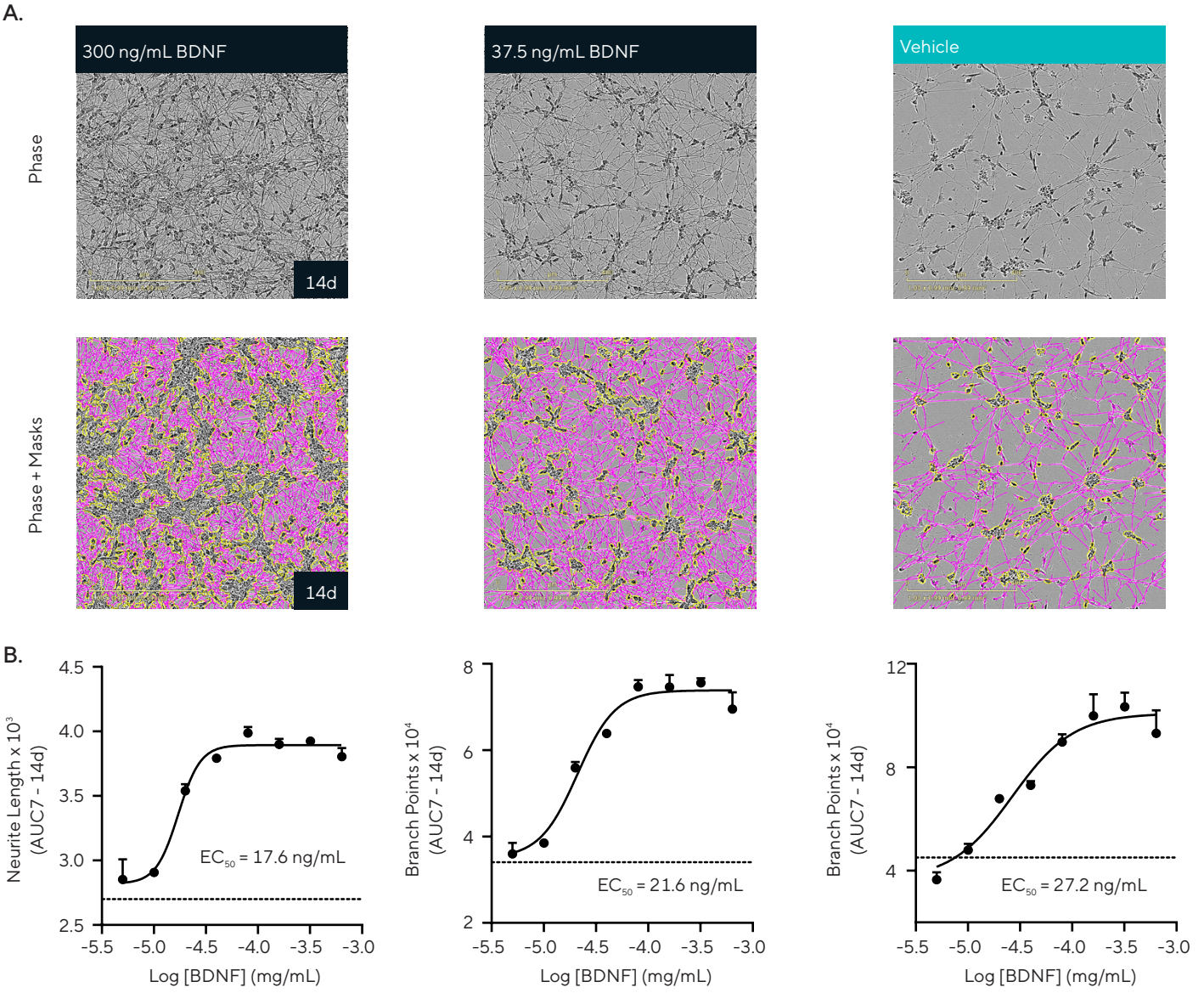

Figure 8. Concentration-dependent neurite development in response to BDNF. SH-SY5Y cells were differentiated by gradual serum starvation (10 – 0%) in combination with atRA (10 µM) and a concentration range of Human Recombinant BDNF (600 – 4.69 ng/mL). Images were acquired at 10X over 14 days using the Incucyte® Live-Cell Analysis System and quantified using integrated Incucyte® Neurotrack Analysis Software. A) HD Phase images for BDNF differentiated neuronal cultures compared to serum-free vehicle control. Label-free segmentation masks of neurites (magenta) and cell-body clusters (yellow outline) also shown. C) Concentration response curves of Neurotrack morphological readouts showing Neurite Length, Neurite Branch Points, and Cell-Body Cluster Area (AUC 7 - 14 days). Data presented as mean ± SEM, n = 3 replicates.

Summary and Outlook

Brain development and differentiation are highly sophisticated, dynamic processes involving a complex interplay of several growth factors and cytokines, which require robust, translational *in vitro* models that accurately recapitulate desired phenotypes. The data shown demonstrates how utilizing Sartorius RUO Growth Factors and Cytokines in combination with live-cell analysis methods is a powerful approach for reproducibly generating *in vitro* neuronal cultures. This approach facilitates optimization of cell culture, or differentiation conditions, and provides an efficient platform to investigate biological mechanisms underlying specific phenomena and to perform pre-clinical screening studies of neurotrophic and neurotoxic agents.

References

1. Shipley MM, Mangold CA, Szpara ML. Differentiation of the SH-SY5Y Human Neuroblastoma Cell Line. *Journal of Visualized Experiments*. 2016;(108). doi:10.3791/53193
2. Zhao B, Schwartz JP. Involvement of cytokines in normal CNS development and neurological diseases: Recent progress and perspectives. *J Neurosci* Res. 1998;52(1):7-16. doi:10.1002/(SICI)1097-4547(19980401)52:1<7::AID-JNR2>3.0.CO;2-I
3. Xiao N, Le QT. Neurotrophic Factors and Their Potential Applications in Tissue Regeneration. *Arch Immunol Ther Exp (Warsz)*. 2016;64(2):89-99. doi:10.1007/s00005-015-0376-4
4. Biedler JL, Helson L, Spengler BA. Morphology and Growth, Tumorigenicity, and Cytogenetics of Human Neuroblastoma Cells in Continuous Culture1. *Cancer Res*. 1973;33(11):2643-2652. http://aacrjournals.org/cancerres/article-pdf/33/11/2643/2390476/cr0330112643.pdf
5. Ross RA, Spengler BA, Biedler JL. Coordinate Morphological and Biochemical Interconversion of Human Neuroblastoma Cells. JNCI: *Journal of the National Cancer Institute*. 1983;71(4):741-747. doi:10.1093/jnci/71.4.741
6. Dwane S, Durack E, Kiely PA. Optimising parameters for the differentiation of SH-SY5Y cells to study cell adhesion and cell migration. *BMC Res Notes*. 2013;6(1):366. doi:10.1186/1756-0500-6-366
7. Kovalevich J, Langford D. Considerations for the use of SH-SY5Y neuroblastoma cells in neurobiology. *Methods in Molecular Biology*. 2013;1078:9-21. doi:10.1007/978-1-62703-640-5_2
8. Cheung YT, Lau WKW, Yu MS, et al. Effects of all-trans-retinoic acid on human SH-SY5Y neuroblastoma as *in vitro* model in neurotoxicity research. *Neurotoxicology*. 2009;30(1):127-135. doi:10.1016/j.neuro.2008.11.001
9. Påhlman S, Ruusala AI, Abrahamsson L, Odelstad L, Nilsson K. Kinetics and concentration effects of TPA-induced differentiation of cultured human neuroblastoma cells. *Cell Differ*. 1983;12(3):165-170. doi:10.1016/0045-6039(83)90006-4
10. Kim B, Leventhal PS, Saltiel AR, Feldman EL. Insulin-like Growth Factor-I-mediated Neurite Outgrowth *in Vitro* Requires Mitogen-activated Protein Kinase Activation. *Journal of Biological Chemistry*. 1997;272(34):21268-21273. doi:10.1074/jbc.272.34.21268
11. Encinas M, Iglesias M, Liu Y, et al. Sequential treatment of SH-SY5Y cells with retinoic acid and brain-derived neurotrophic factor gives rise to fully differentiated, neurotrophic factor-dependent, human neuron-like cells. *J Neurochem*. 2000;75(3):991-1003. doi:10.1046/j.1471-4159.2000.0750991.x

12. Ferlemann FC, Menon V, Condurat AL, Rößler J, Pruszak J. Surface marker profiling of SH-SY5Y cells enables small molecule screens identifying BMP4 as a modulator of neuroblastoma differentiation. *Sci Rep*. 2017;7(1):13612. doi:10.1038/s41598-017-13497-8
13. Katsetos CD, Herman MM, Mörk SJ. Class III β-tubulin in human development and cancer. *Cell Motil Cytoskeleton*. 2003;55(2):77-96. doi:10.1002/cm.10116
14. Påhlman S, Ruusala AI, Abrahamsson L, Mattsson MEK, Esscher T. Retinoic acid-induced differentiation of cultured human neuroblastoma cells: a comparison with phorbolester-induced differentiation. *Cell Differ*. 1984;14(2):135-144. doi:10.1016/0045-6039(84)90038-1
15. Kawabata M. Signal transduction by bone morphogenetic proteins. *Cytokine Growth Factor Rev*. 1998;9(1):49-61. doi:10.1016/S1359-6101(97)00036-1
16. Wang RN, Green J, Wang Z, et al. Bone Morphogenetic Protein (BMP) signaling in development and human diseases. *Genes Dis*. 2014;1(1):87-105. doi:10.1016/j.gendis.2014.07.005
17. Chen D, Zhao M, Mundy GR. Bone Morphogenetic Proteins. *Growth Factors*. 2004;22(4):233-241. doi:10.1080/08977190412331279890
18. Gilliam DT, Menon V, Bretz NP, Pruszak J. The CD24 surface antigen in neural development and disease. *Neurobiol Dis*. 2017;99:133-144. doi:10.1016/j.nbd.2016.12.011
19. Bekinschtein P, von Bohlen und Halbach O. Editorial: Cellular and Molecular Mechanisms of Neurotrophin Function in the Nervous System. *Front Cell Neurosci*. 2020;14. doi:10.3389/fncel.2020.00101
20. Chen BY, Wang X, Wang ZY, Wang YZ, Chen LW, Luo ZJ. Brain-derived neurotrophic factor stimulates proliferation and differentiation of neural stem cells, possibly by triggering the Wnt/β-catenin signaling pathway. *J Neurosci Res*. 2013;91(1):30-41. doi:10.1002/jnr.23138
21. 2Brafman DA. Generation, Expansion, and Differentiation of Human Pluripotent Stem Cell (hPSC) Derived Neural Progenitor Cells (NPCs). In: *Stem Cell Renewal and Cell-Cell Communication*. Vol 1212. Springer New York; 2014:87-102. doi:10.1007/7651_2014_90
22. Jacob F, Pather SR, Huang WK, et al. Human Pluripotent Stem Cell-Derived Neural Cells and Brain Organoids Reveal SARS-CoV-2 Neurotropism Predominates in Choroid Plexus Epithelium. *Cell Stem Cell*. 2020;27(6):937-950. doi:10.1016/j.stem.2020.09.016
23. Agholme L, Lindström T, Kågedal K, Marcusson J, Hallbeck M. An *In Vitro* Model for Neuroscience: Differentiation of SH-SY5Y Cells into Cells with Morphological and Biochemical Characteristics of Mature Neurons. *Journal of Alzheimer's Disease*. 2010;20(4):1069-1082. doi:10.3233/JAD-2010-091363
24. Teppola H, Sarkanen JR, Jalonen TO, Linne ML. Morphological Differentiation Towards Neuronal Phenotype of SH-SY5Y Neuroblastoma Cells by Estradiol, Retinoic Acid and Cholesterol. *Neurochem Res*. 2016;41(4):731-747. doi:10.1007/s11064-015-1743-6

North America
Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
USA
Phone +1 734 769 1600
Email: orders.US07@sartorius.com

Europe
Sartorius UK Ltd.
Longmead Business Centre
Blenheim Road
Epsom
Surrey, KT19 9QQ
United Kingdom
Phone +44 1763 227400
Email: eurordersUK03@sartorius.com

Asia Pacific
Sartorius Japan K.K.
4th Floor, Daiwa Shinagawa North Bldg.
1-8-11, Kita-Shinagawa 1-chome
Shinagawa-Ku
Tokyo 140-0001
Japan
Phone +81 3 6478 5202
Email: eurordersUS07@sartorius.com

Find out more: www.sartorius.com/incucyte
www.sartorius.com/ruo

For questions, email: AskaScientist@sartorius.com

Publication No.: Optimization-SH-SY5Y-Application-Note-en-Sartorius
Status: 07 | 2023