Application Note

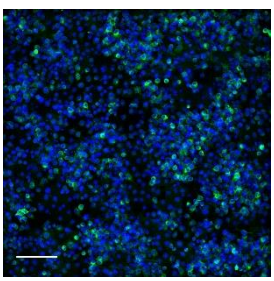
PODS™ Human Wnt-3a (Cat PPH300)

Neuronal induction in rat primary hippocampal neuronal progenitor cells

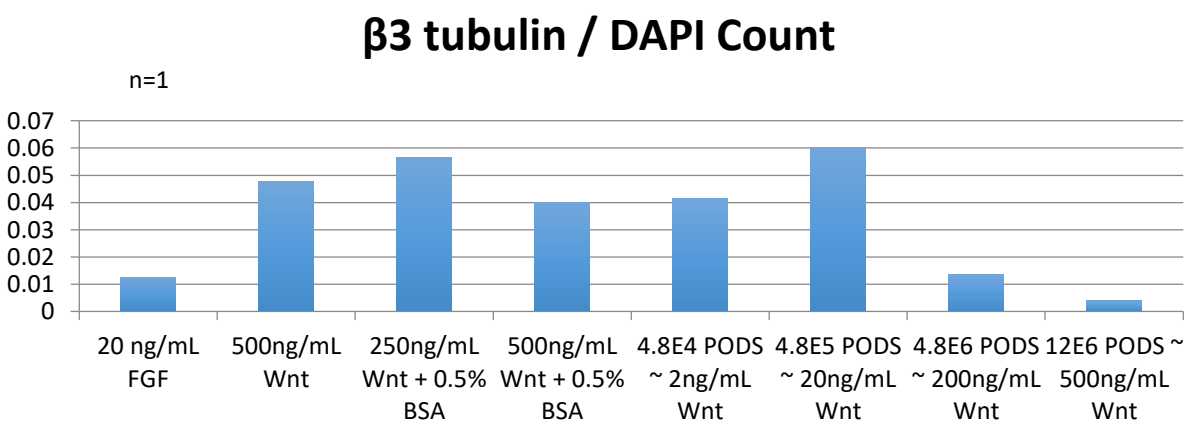
Data Courtesy of Riya Muckom and David Schaffer, UC Berkeley

Issued June 9th 2017

Cell type	primary adult hippocampal neural progenitor cells from rat
Culture format	96-well plate coated with 10 µg/ml p-ornithine then 5µg/ml laminin
Cell seeding density	2.5E4 cells/cm^2 (3E4 cells/ml)
Media replenishment	Non-PODS™: half media change daily for 6 days PODS™: no media change for 6 days
Immunocytochemistry	β3-tubulin: Sigma T-8578, 1:1000 dilution Hoechst 1:2000 dilution



Cells cultured with 4.8E4 PODS™/ml



Assay results Equivalent PODS™ Wnt-3a protein concentrations were calculated assuming 50 million PODS™ crystals generates a peak available concentration of 3.3 µg. 20 ng of PODS™ Wnt-3a gave the same neuronal conversion efficiency as 250 ng of standard Wnt-3a. Inhibition of neuron formation was observed at higher PODS™ Wnt-3a concentrations.

Comments Combining this factor with reduced media changes, PODS™ Wnt-3a costs were 97% lower, or less than 1/20th of standard Wnt-3a. In other experiments, empty PODS™ have been shown to have no effect. Inhibition at high PODS™ concentrations may be due to over-stimulation of wnt signaling pathways or toxic effects of Wnt-3a. Lower equivalent concentrations of PODS™ Wnt-3a out-perform standard Wnt-3a.

Note This experiment was the first attempt and performed without any optimization for PODS™. Introducing one or two half media changes (without any additional PODS™ crystals, may improve efficacy further.