

Multiplexing the Developmental Neurotoxicity *In Vitro* Battery Proliferation and Apoptosis Endpoints Into An Optimized 384-Well Assay

Gabrielle Byrd^{1,2}, Theresa Freudenrich¹, Seline Choo^{1,2}, Kathleen Wallace¹, Kelly Carstens¹, Megan Culbreth^{1,3}, Timothy Shafer¹, Joshua Harrill¹

¹Center for Computational Toxicology and Exposure, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, USA

²Oak Ridge Institute for Science and Education (ORISE), Oak Ridge, Tennessee, USA

³Human Foods Program, U.S. Food and Drug Administration, Laurel, Maryland, USA

Background

The Developmental Neurotoxicity In Vitro Battery (DNT-IVB) is a set of 17 assays that assess critical processes of nervous system development.

Currently, the DNT-IVB assesses proliferation and apoptosis in human neural progenitor (hNP1) cells using two separate 96-well format assays.

To increase the efficiency of chemical testing, we have developed a multiplexed 384-well high content imaging assay that assesses proliferation and apoptosis simultaneously in hNP1 cells.

Procedure

Plate Cells

Dose Cells

Live-Label and Fix Cells

Immunocytochemistry

Imaging and Analysis

48-hrs

20-hrs

4-hrs

72-hrs

24-hrs

Certus Flex
Echo 550
MultiFlo FX
Opera Phenix

For more
detailed
protocol
information,
feel free to
request the
supplemental
file!

Novel, Multiplexed, 384-Well Proliferation and Apoptosis Assay Performs Favorably As Compared to Original 96-Well Proliferation and Apoptosis Assays

Chemical	Proliferation		Apoptosis	
	384 Well AC50 (μM)	96 Well AC50 (μM)	384 Well AC50 (μM)	96 Well AC50 (μM)
Aphidicolin	3	10 ^{a, †}	--	-- ^{a, †}
5-fluorouracil	3.7	4.62 ^b	8.1	23.78 ^b
Cytosine arabinoside	0.07	0.08 ^b	0.66	0.21 ^b
Staurosporine	0.03	No Data	0.17	0.1 ^{a, †}
Hydroxyurea	96	-- ^b	--	No Data
Caffeine	--	-- ^b	--	-- ^c
Ketamine	--	-- ^b	--	-- ^c
Saccharin	--	-- ^c	--	-- ^c
Sorbitol	--	-- ^c	--	-- ^c

Table 1. Comparison of AC50s for 384-Well and Original 96-Well Assay Endpoints for Compounds Used to Optimize the 384-Well Assay. Two dashes (--) indicate inactivity. AC50s are the concentration at half maximal activity. ^a Culbreth et al, 2012 | ^b CompTox Chemicals Dashboard (v2.4.1; <https://comptox.epa.gov/dashboard>) | ^c Harrill et al, 2018 | [†] ReNcell® CX cells, no data available in hNP1 cells.

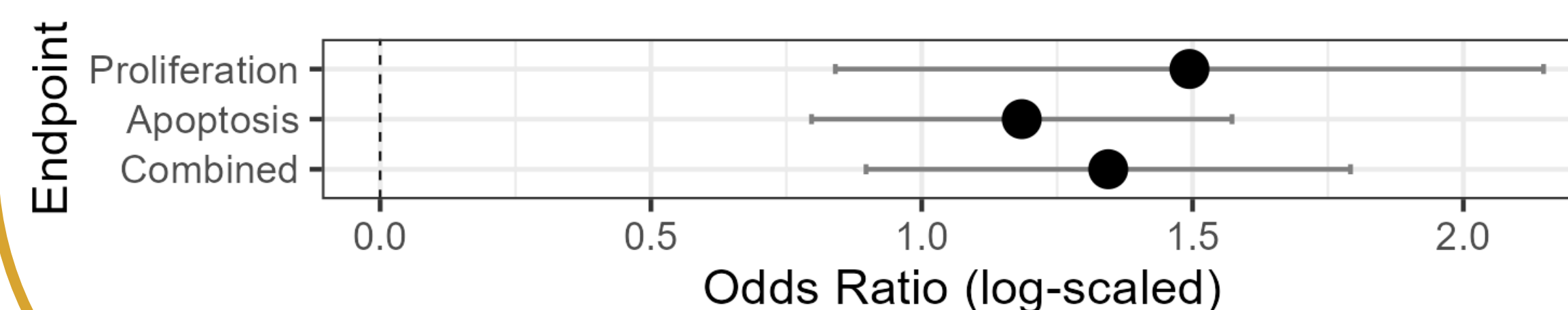


Figure 2. Odds Ratio Analysis Evaluating Capability of the 384-Well Assay to Predict Hits in the 96-Well Assays. For the proliferation endpoint, the 384-well assay is roughly 31 times as likely to identify a chemical as active if it is active in the 96-well assay as opposed to inactive in the 96-well assay. It is approximately 15 times as likely and approximately 22 times as likely for the apoptosis endpoint and combined endpoints, respectively. The odds ratio analysis is visualized on the left for all three endpoints with log-scaled odds and 95% confidence interval values. The contingency tables utilized in the odds ratio analysis calculations (normal approximation) are represented on the right.

	Proliferation			Apoptosis			Combined	
	384-Well +	384-Well -		384-Well +	384-Well -		384-Well +	384-Well -
96-Well +	20	2	96-Well +	27	9	96-Well +	34	5
96-Well -	33	103	96-Well -	20	102	96-Well -	28	91

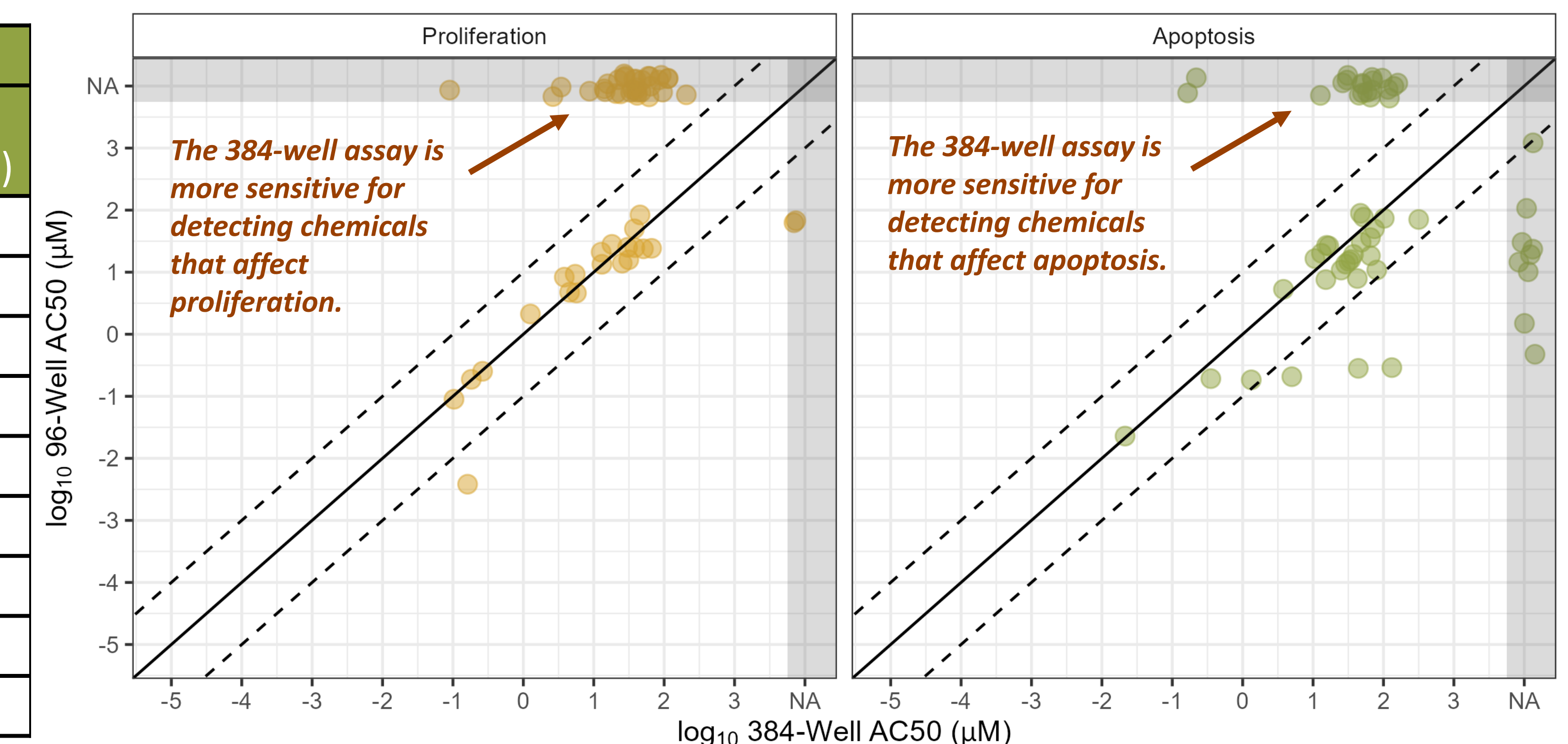


Figure 1. Comparison of AC50s for 384-Well and Original 96-Well Assay Endpoints for a Set of 56 DNT-Relevant Chemicals. Each point represents the AC50 values for a given compound, with proliferation data colored in gold and apoptosis data colored in green. The solid line is the unity line and is surrounded by dashed lines representing a deviation of one order of magnitude. Grey boxes on the plot edges contain inactive compounds. Compounds inactive across all endpoints are excluded.

Future Directions

- Incorporating this assay into the DNT-IVB.
- Optimizing and multiplexing 384-well assays for DNT-IVB neurite outgrowth and synaptogenesis assays previously developed by the US-EPA.

References

Culbreth ME, Harrill JA, Freudenrich TM, Mundy WR, Shafer TJ (2012) Comparison of chemical-induced changes in proliferation and apoptosis in human and mouse neuroprogenitor cells. *Neurotoxicology* 33:6. doi: 10.1016/j.neuro.2012.05.012
Harrill JA, Freudenrich T, Wallace K, Ball K, Shafer TJ, Mundy WR (2018) Testing for developmental neurotoxicity using a battery of *in vitro* assays for key cellular events in neurodevelopment. *Toxicology and Applied Pharmacology* 354. doi: 10.1016/j.taap.2018.04.001
Mundy WR, Padilla S, Breier JM, Crofton KM, Gilbert ME, Herr DW, Jensen KF, Radio NM, Raffaele KC, Schumacher K, Shafer TJ and Cowden J (2015). Expanding the test set: Chemicals with potential to disrupt mammalian brain development. *Neurotoxicology and Teratology* 52. doi: 10.1016/j.ntt.2015.10.001
Martin MM, Baker NC, Boyes WK, Carstens KE, Culbreth ME, Gilbert ME, Harrill JA, Nyffeler J, Padilla S, Friedman KP and Shafer TJ (2022). An expert-driven literature review of "negative" chemicals for developmental neurotoxicity (DNT) *in vitro* assay evaluation. *Neurotoxicology and Teratology* 93. doi: 10.1016/j.ntt.2022.107117