

**Cellular identity** in microBrain<sup>®</sup> Assay Ready cultures was verified using immunocytochemical analysis to determine cell type, demonstrate complex synaptic interconnections, and confirm manufacturing batch reproducibility.

microBrain 2D and microBrain 3D are physiologically relevant and arrive preplated and assay ready.

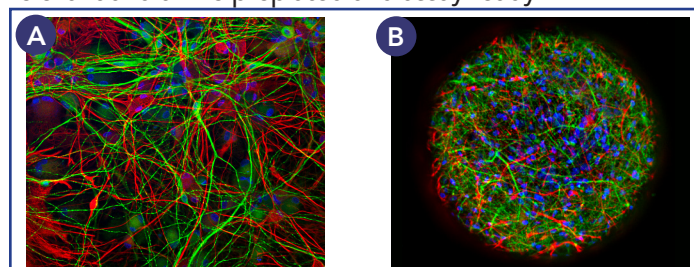


Figure 1. (A) microBrain 2D and (B) microBrain 3D neural cultures are comprised of a coculture of neurons (MAP2) and astrocytes (GFAP). Nuclei stained with DAPI.

**Quantification by differential immunofluorescence** in microBrain cultures demonstrated approximately equivalent numbers of neurons and astrocytes.

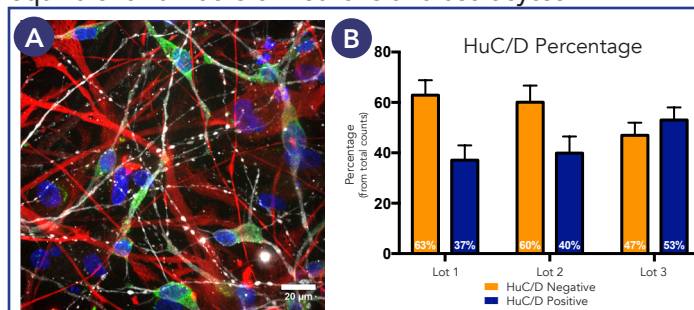


Figure 2. (A) Colocalization of HuC/D with MAP2 but not with GFAP in microBrain 2D. (B) Comparison of HuC/D positive and negative cell populations across three lots. Nuclei stained with DAPI.

**Immunocytochemical staining of microBrain 2D** revealed a neural composition of glutamatergic and GABAergic neurons at approximately 90% and 10%, respectively.

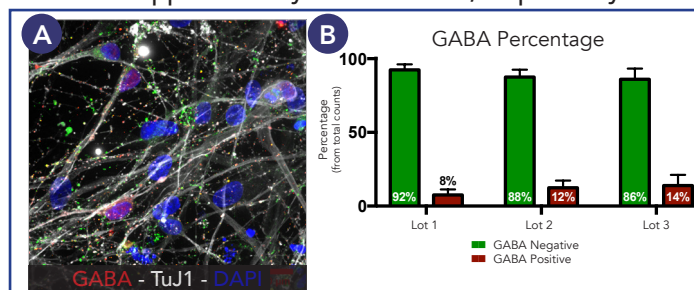


Figure 3. (A) Identification of GABAergic neurons with TuJ1, GABA, and DAPI in microBrain 2D. (B) Quantification of GABA positive and negative cell populations across three lots.

**Mature synapses** were identified by colocalization of pre- and post-synaptic markers.

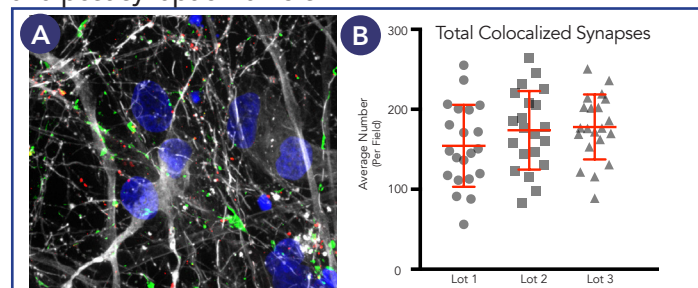


Figure 4. (A) TuJ1 labeling with Syn1 (pre-synaptic) and PSD95 (post-synaptic) indicating mature synapses. (B) Total colocalized synapses per field across three lots.

microBrain 3D spheroids show uniform distribution of neurons and astrocytes throughout the spheroids as well as the absence of central necrosis.

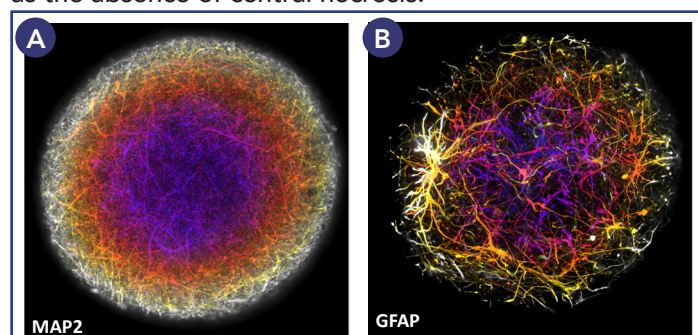


Figure 5. Pseudo-coloring to indicate z-axis depth illustrates (A) neuron and (B) astrocyte distribution throughout the spheroid.

#### microBrain 2D and 3D

- Show a cellular composition similar to that of the native human cortex.
- Express proteins known to be markers of synaptic maturity.
- Provide a relevant and robust human cortical CNS model for high throughput drug discovery and toxicology studies.

For more information on this or other microBrain characterization data, please visit [StemoniX.com](http://StemoniX.com) and contact us at [info@stemonix.com](mailto:info@stemonix.com)