



# ENStem™-A Human Neural Progenitors provide a user friendly source of human embryonic stem cell derived adherent neural cells

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A renewable source of physiologically relevant human primary neural cells would assist basic researchers and drug discovery by providing material for varied experimental paradigms and assays. Importantly, if these cells could grow in an adherent monolayer, then large numbers of neural cells derived from a single source could be used for characterizing aspects of neuroprotection, neurite outgrowth, synaptogenesis, growth cone collapse, the viability of neural cells (cell health, genotoxicity, cell cycle regulation, DNA repair), pathways (phenotypic differentiation, intracellular signaling) and pharmacology (dose response relationships, receptor desensitization).

## Background

Most neurons are produced during embryonic development by neural stem cells or neural progenitor cells. Aruna Biomedical Inc has licensed from the University of Georgia a method to derive neural progenitors from NIH approved human embryonic stem cells (hESCs) (Shin *et al.* 2006).

These Neural Progenitor Cells have been exclusively licensed by Millipore. The novelty of this product is that researchers can use a kit containing cryopreserved hESC derived neural progenitors along with required media to

propagate and differentiate primary cultures of neural cells. The cells and kit are available in a convenient ready to use format that is marketed by Millipore under the ENStem-A brand name.

The innovative adherent ENStem-A cell culture system enhances the means to reliably differentiate these cells for quantitative studies of neurons and glial cells, and will help neurobiologists understand the function of these cells. Many existing sources of human neural progenitors are propagated as suspensions of neurospheres that may not be easily adapted for some studies. ENStem-A cells grown in a monolayer are amenable to studies involving electrophysiology, axon guidance and other quantitative analysis mentioned above or for use in high-throughput, and high-content screens for therapeutic compounds or for tests of neurotoxicity.

## Uniformity of ENStem-A cells

The adherent ENStem-A cells were derived from WA09 (H9) hESC and these lines are greater than 90 % positive for neural stem cell markers and less than 5 % of the cells remained hESC-marker positive. Both flow cytometry (Table 1) and microscopy were used to quantify these levels (Figure 1). After differentiation to neural progenitors, these

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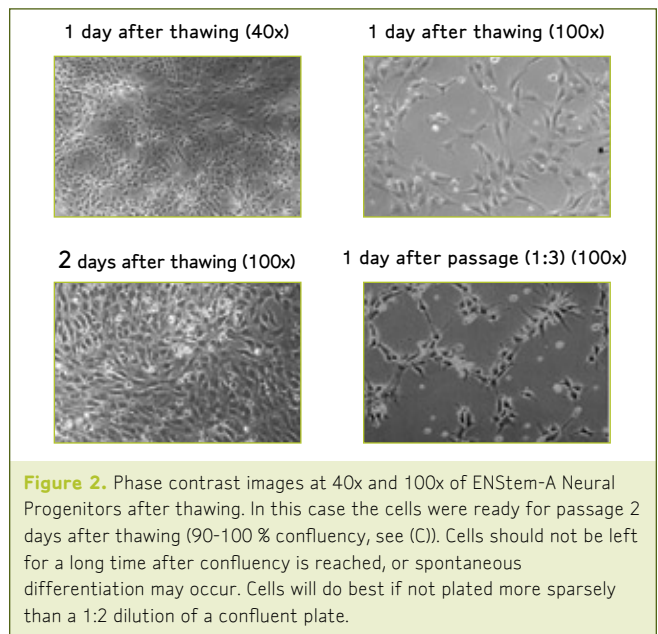
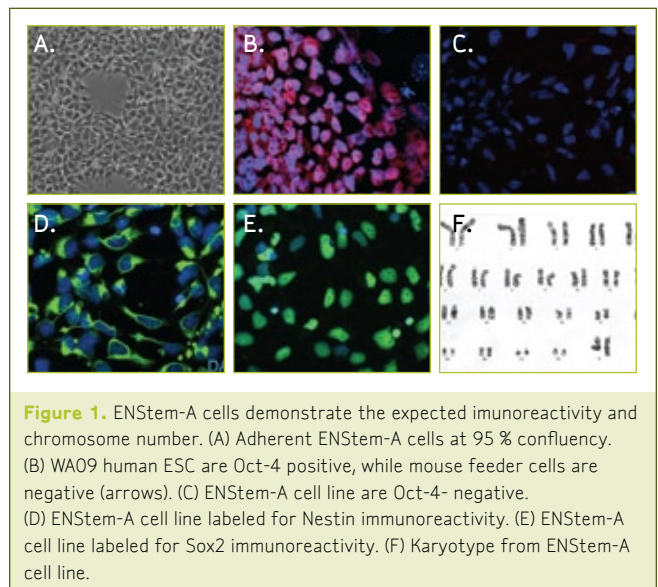
cells had a normal complement of chromosomes (euploidy) following ten passages (Figure 1). Although adult, fetal and hESC-derived neurospheres can be differentiated into primary cultures of neurons and glial cells the 3-dimensional aspects of the spheres complicate access to many experimental treatments. The ENStem-A Neural Progenitor Expansion kit (Cat. No. SCRO55) contains cells and proliferation media to propagate the cells. This is a defined media containing LIF and FGF2, however, BMP4 inhibitor factors (Noggin) or conditioned media are not required. These cells have a population doubling time of approximately 24 hours when grown at high density (Figure 2). The uniformly adherent cultures of ENStem-A cells are likely to respond reproducibly to a wide variety of experimental conditions.

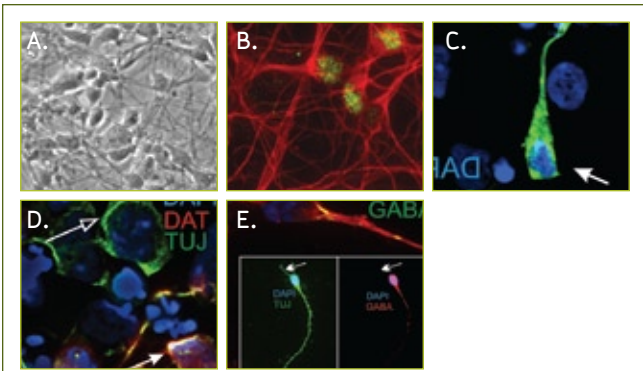
## DIFFERENTIATION OF ENSTEM-A CELLS

The ENStem-A cells are useful for researchers who may want to or have already developed their own differentiation protocols (i.e. induction of dopaminergic cells using SHH and FGF8 or motoneurons using SHH and RA). ENStem-A cells can produce multiple neuronal phenotypes (Figure 3) and glial phenotypes (Figure 4) (also see Shin, *et al*, 2006) that would be relevant to multiple disease models of the nervous system (Table 2). Use of growth factors and/or genetic markers will no doubt be useful for enrichment of neural cell phenotypes and ENStem-A cells are amenable to gene insertions. We have previously published that SHH and RA can be used to induce motoneuron differentiation in our ENStem-A cells<sup>2</sup>. Finally, we have maintained differentiated cultures for up to 4 months and demonstrated that these cultures begin to form a plexus of cells, demonstrating that they behave similar to primary cell types. We have used immunofluorescence analysis to quantify recovery of neurons after using ENStem-A differentiation medium. ENStem-A cells were plated in 4-well chamber slides coated with poly-ornithine and laminin for 7 and 14 days in differentiation medium without bFGF and LIF. LIF was

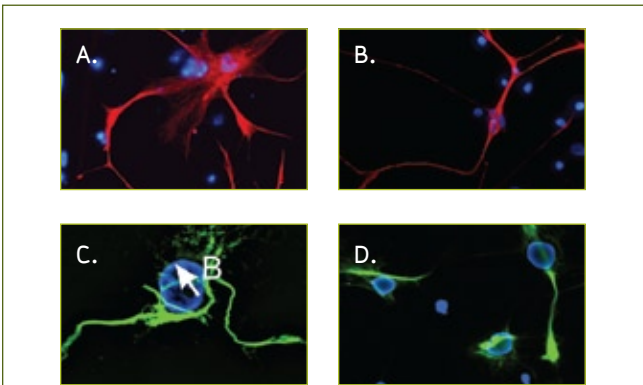
Cell Line	Using immunofluorescence (>1000 cells counted)			Using flow cytometry (>10,000 cells counted)	
	% nestin <sup>+</sup>	% SOX2 <sup>+</sup>	% OCT4 <sup>+</sup>	% nestin <sup>+</sup>	% OCT4 <sup>+</sup>
ENStem-A	98 ± 1	94 ± 2	2 ± 0.51	99.4	0.34
Renal				.5	NA
HUVEC				NA	0.01

**Table 1.** % positive ENStem-A cells using immunofluorescence or flow cytometry

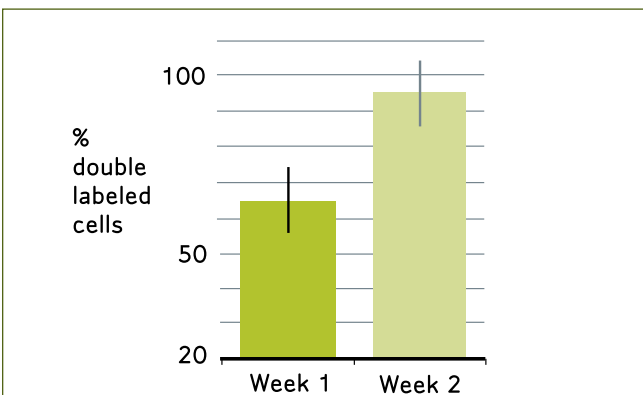




**Figure 3.** Neural phenotypes derived from ENStem-A cell lines. (A) Phase contrast image of differentiated culture. (B) Network including post-mitotic motoneurons (HB9). (C) Cholinergic neuron. (D)  $\beta$ III Tubulin (Tuj-1) positive cells that are DAT-positive (dopamine transporter; closed arrow) and DAT-negative (open arrow). (E) Gabaergic neurons, inset illustrates GABA in axon but not the dendrites (arrow).



**Figure 4.** Glial cells. (A and B) GFAP-positive cells. (A) represents the morphological characteristics of astrocytes whereas (B) represents the structure of radial glia. (C and D) mature oligodendrocytes are defined by expressing myelin basic protein.



**Figure 5.**  $\beta$ III Tubulin (Tuj-1) positive (neural) cells after 1 week and 2 weeks without proliferative factors (% double labeled cells = Tuj-1 and DAPI positive).

removed to attempt to preferentially differentiate the cells to a neural phenotype. Projected images of 5 random fields of ~15 cells from each replicate were scored by inspection for the frequency of cells showing immunostaining of the indicated neuronal marker  $\beta$ III Tubulin (Tuj-1). The average percentage of Tuj-positive cells was 65 % after 7 days and 96 % after 14 days (Figure 5). Further refinements should lead to methods that generate an enriched population of neurons for research. However, these enriched neural populations do have a high cell death rate and this can be reduced by adding growth factors such as LIF, GDNF and BDNF to the differentiation media.

## CONCLUSION

ENStem-A cell monolayer cultures are uniform, robust, easily propagated and can differentiate into many neural lineages. Millipore, in conjunction with Aruna Biomedical Inc., offers neurobiologists a normal, non-transformed hESC derived source to study the diverse function, growth and differentiation of human neuronal cells.

Marker for cellular phenotype	Pathology related to phenotype
<b>ChAT</b> (Choline Acetyltransferase) – synthetic enzyme	Alzheimer’s disease
<b>HB9</b> - transcription factor marker in motoneurons (cholinergic)	ALS, spinal injury, SMA
<b>Dopamine transporter (DAT)</b>	Parkinson’s disease, schizophrenia, depression, addictive disorders
<b>GABA</b> – transmitter	Pain processing, conscious state (anesthesiology), alcohol research

**Table 2.** Neural phenotypes derived from ENStem-A cell lines and their relevance to disease research.

## References

1. Shin et al. (2005). Stem Cells **24** 125-38.
2. Shin et al. (2006). Stem Cells Dev **14** 66-9.



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