

# COLONY ASSAY FOR NEURAL STEM AND PROGENITORS ADAPTED TO 24 AND 96-WELL PLATE FORMATS

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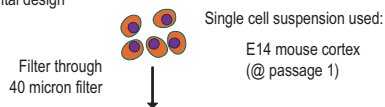
## Introduction

Reynolds and Weiss were first to report that a subset of CNS cells have the ability to proliferate in culture over multiple passages (Reynolds and Weiss, Dev Bio 1996). The spheroid clusters of cells generated in these liquid cultures were termed neurospheres and the numbers of neurospheres produced after two culture passages (the Neurosphere Assay – NA) used as quantitative measure of neural stem cell (NSC) frequency. In recent years several publications have reported significant limitations of the NA as a measure of NSC potential. Reynolds and Rietze (Nature 2005) have shown not all cells capable of forming a neurosphere meet the criteria for a NSC, which include the ability to self-renew, produce large number of progeny and differentiate into the three main neural cell lineages. Neurospheres are not clonally derived and fusion of neurospheres occurs in >40% of the neurospheres in suspension cultures (Singec et al, Nature Methods 2006). Finally, differences in neurosphere culture conditions and procedures prevent direct comparison of results between labs (Chaichana et al, PLOs, 2006).

Previously we developed the Neural Colony Forming Cell (NCFC) assay, which discriminates between NSCs and progenitors by the colonies they form in semi-solid media. In the NCFC assay, colonies >2 mm in diameter are derived from cells that meet the criteria for a NSC and are referred to as NCFC-NSC, while cells that form colonies <2 mm in size, lack self-renewal ability and are referred to as NCFC-progenitor (Louis et al, Soc for Neurosci. 2004). We recently provided evidence that the cells which form spheres in the NA will also read out in the NCFC assay by forming a colony. However, 99% of the colonies generated in the NCFC assay are clonally derived and typically only 80% of neurospheres (NA) are clonally derived. The objective of this study was to miniaturize the NCFC assay from the standard 35 mm dish format to 24- or 96-well formats and compare assay reproducibility and linearity with cell plating density.

## Methods

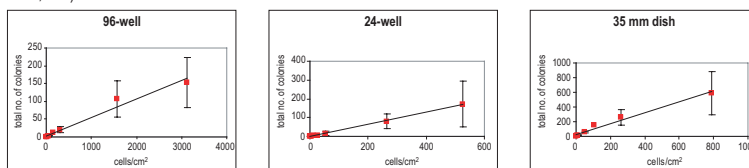
Figure 1. Experimental design



Neural Colony Forming Cell (NCFC) Assay									
Media	NeuroCult <sup>®</sup> NCFC assay media (#05740) + 20 ng/mL EGF								
Volume	0.1 mL/96 well 0.3 mL/24 well 1.5 mL/35 mm dish								
Cell density tested	<table border="1"> <thead> <tr> <th>Plate format</th> <th>Cells/well or dish (cells/cm<sup>2</sup>)</th> </tr> </thead> <tbody> <tr> <td>96-well</td> <td>1, 5, 10, 20, 50, 100, 500, 1000 (3, 16, 31, 63, 156, 313, 1563, 3125)</td> </tr> <tr> <td>24-well</td> <td>1, 5, 10, 20, 50, 100, 500, 1000 (1, 3, 5, 11, 26, 53, 263, 526)</td> </tr> <tr> <td>35 mm</td> <td>20, 50, 100, 500, 1000, 2500, 7500 (2, 5, 11, 53, 105, 263, 789)</td> </tr> </tbody> </table>	Plate format	Cells/well or dish (cells/cm <sup>2</sup> )	96-well	1, 5, 10, 20, 50, 100, 500, 1000 (3, 16, 31, 63, 156, 313, 1563, 3125)	24-well	1, 5, 10, 20, 50, 100, 500, 1000 (1, 3, 5, 11, 26, 53, 263, 526)	35 mm	20, 50, 100, 500, 1000, 2500, 7500 (2, 5, 11, 53, 105, 263, 789)
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	<p>Score colonies</p>								

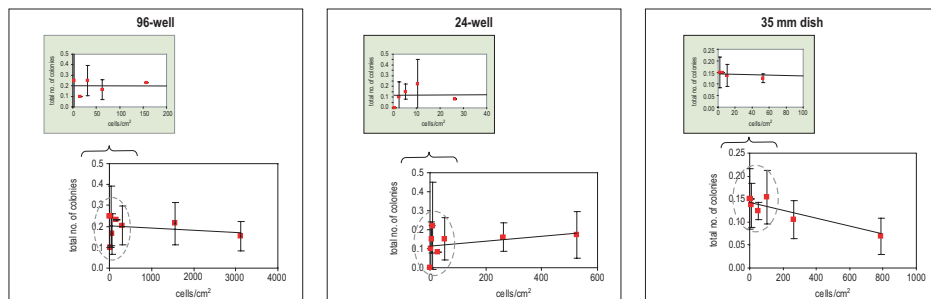
## Results

Figure 2. Relationship between the total number of colonies and the number of cells plated (either cells/well or dish or cells/cm<sup>2</sup>) in three plate formats (mean±SD; n=3)



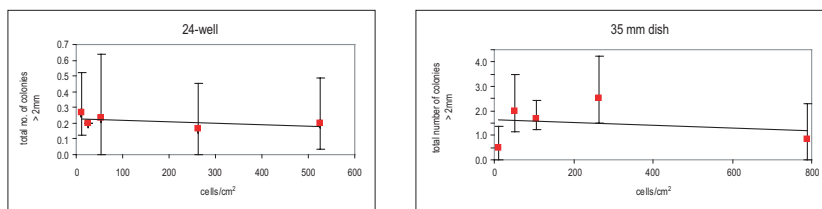
- The relationship between number of colonies and number of cells plated (or cells/cm<sup>2</sup>) was linear for all three formats.

Figure 3. Correlation between the colony output per cells plated and plating density (cells/cm<sup>2</sup>) in three plate formats (mean±SD; n=3)



- There was no significant difference in the maximum colony output per cells plated for the 96-, 24-well and 35 mm dish formats (p>0.05 t-test).

Figure 4. >2mm colony (NCFC-NSC) output versus plating density in 24-well and 35mm plate formats (mean±SD; n=3)



- In 35 mm dishes, the optimal > 2 mm colony output was obtained at 2500 cells/dish (263 cells/cm<sup>2</sup>).
- For 24-wells, optimal NCFC-NSC output was achieved at all plating densities tested.
- The large colonies > 2mm in diameter (NCFC-NSC) were not detected in 96-wells at all densities tested possibly due to the small surface area of the wells.

## Summary

- In terms of total colonies, the NCFC assay can be miniaturized to 24- and 96-well formats, thus providing a more cost-effective high-throughput assay for assessing effects of drugs and factors on NSCs and progenitors.
- However, the rare larger colony > 2mm (formed by NCFC-NSC) cannot be measured in the 96-well format.