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Distinct populations of forebrain neural stem and progenitor cells can be isolated using side-population analysis.

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The absence of stem cell-specific markers has posed challenges to the identification and isolation of stem cells. We report the isolation of a discrete and highly enriched population of neural stem cells from clonally derived colonies of neural stem cell and progenitor cells (neurospheres) after exposure to the fluorescent DNA binding dye Hoechst 33342 and subsequent analysis via dual wavelength flow cytometry. The low fluorescent side population comprised only 3.6% of all live cells sorted yet contained >99% of all the neural stem cells as assayed by the formation of neurospheres in culture. Most neurosphere-derived cells are progenitor cells, and these are found within the higher fluorescence (non-side population) fraction. The isolation of a highly enriched population of self-renewing, multipotential neural stem cells was seen from both adult- and embryonic-derived neurospheres; however, the relative percentage of cells comprising the side-population and the mechanism of dye efflux varied between adult and embryonic donor tissue. Combining the side-population analysis with markers recently shown to enrich for neural stem cells afforded no further enrichment in the case of peanut agglutinin expression and size criteria; however, when the side-population analysis was combined with Lewis X (LeX) expression, a slight enrichment was seen over side-population analysis alone.

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