

## RHB-A<sup>®</sup>

**Catalogue Number** SCS-SF-NB-01

**Size** 500 mL

**Applications** Demonstrated applications of RHB-A include:

- Maintenance and expansion of adherent, mouse and human NS cells
- Neural differentiation of mouse embryonic stem (ES) cells in monolayer culture
- Derivation of mouse and human NS cells from ES cells and fetal/adult tissues
- Differentiation of mouse and human NS cells into functional neurons
- Maintenance and expansion of human glioblastoma stem cell lines

**Description**

RHB-A, supplemented with Epidermal Growth Factor (EGF) and Fibroblast Growth Factor-2 (FGF-2), enables the maintenance and continual expansion of symmetrically-dividing NS cells<sup>1-5,9</sup> in defined, serum-free adherent culture. In growth factor supplemented RHB-A, NS cells have been demonstrated to retain their neurogenic capacity for over 100 generations, with full maintenance of diploid karyotype<sup>1</sup>. RHB-A in the presence of EGF and FGF-2 also supports the derivation of clonogenic NS cell lines<sup>1</sup>.

RHB-A is significantly more effective than N2B27<sup>4</sup> at promoting the improved differentiation of mouse embryonic stem cells into neurons without the need for growth factor supplementation<sup>7,8</sup>.

Culture of adherent NS cells in RHB-A with sequential growth factor withdrawal leads to differentiation into functional neurons<sup>1</sup>.

RHB-A supplemented with EGF and FGF-2 has recently been used for the propagation of glioblastoma stem cell lines<sup>6</sup>.

*In vitro* human brain organotypic primary cell cultures with expansion and differentiation of radial glia derived NS cells<sup>9</sup>.

**Storage**

Upon receipt, store at -20°C until ready to use. When stored under these conditions the product is stable for 6 months from the date of manufacture (see label).

Once thawed, store at 4°C and use within 4 weeks.

**This product is light sensitive, and should be protected from light.**

**Preparation**

For NS cell propagation and derivation, supplement RHB-A with EGF and FGF-2 (not supplied).

For monolayer differentiation of mouse ES cells into neural precursors, RHB-A is complete, ready-to-use, and does not require growth factor supplementation.

**Quality control**

SC Proven<sup>®</sup> media products undergo rigorous quality control procedures before release.

## References

1. Sun Y, Pollard S, Conti L, Toselli M, Biella G, Parkin G, Willatt L, Falk A, Cattaneo E, Smith A (2008). Long-term tripotent differentiation capacity of human neural stem (NS) cells in adherent culture. *Molecular and Cellular Neuroscience* **38**:245-258
2. Conti L, Pollard SM, Gorba T, Reitano E, Toselli M, Biella G, Sun Y, Sanzone S, Ying QL, Cattaneo E, Smith A (2005). Niche-Independent symmetrical self-renewal of a mammalian tissue stem cell. *PLoS Biology* **3(9)**: e283
3. Pollard SM, Conti L, Sun Y, Goffredo D, Smith A (2006). Adherent Neural Stem (NS) cells from fetal and adult forebrain. *Cerebral Cortex* **16**:112-120
4. Ying QL, Stavridis M, Griffiths D, Li M, Smith A (2003). Conversion of embryonic stem cells into neuroectodermal precursors in adherent monoculture. *Nature Biotechnology* **21**:183-186
5. Pollard SM, Wallbank R, Tomlinson S, Grotewold L, Smith A (2008). Fibroblast growth factor induces a neural stem cell phenotype in foetal forebrain progenitors and during embryonic stem cell differentiation. *Molecular and Cellular Neuroscience* **38**:393-403
6. Pollard SM, Yoshikawa K, Clarke ID, Danovi D, Stricker S, Russell R, Bayani J, Head R, Lee M, Bernstein M, Squire J, Smith A, Dirks P (2009). Glioma stem cell lines expanded in adherent culture have tumor-specific phenotypes and are suitable for chemical and genetic screens. *Cell Stem Cell* **4**:568-580
7. Diogo MM, Henrique D, Cabral JM (2008). Optimization and integration of expansion and neural commitment of mouse embryonic stem cells. *Biotechnology and Applied Biochemistry* **49**:105-112
8. Abranches E, Silva M, Pradier L, Schulz H, Hummel O, Henrique D, Bekman E (2009). Neural differentiation of embryonic stem cells *in vitro*: A road map to neurogenesis in the embryo. *PLoS ONE* **4(7)**: e6286
9. Hansen DV, Lui JH, Parker PRL, Kriegstein AR (2010). Neurogenic radial glia in the outer subventricular zone of human neocortex. *Nature* **464(7288)**:554-561

## Recommended Use

### Maintenance of mouse NS and human NS cell lines

When supplemented with EGF and FGF-2, RHB-A medium supports the growth and maintenance of both adherent mouse NS cells and human NS cells in serum-free conditions<sup>1,5</sup> (NOTE: The recommended concentrations of EGF & FGF-2 are 10 - 20ng/mL. However, these should be optimized by the end user). The medium should be changed every 2 - 3 days and cell plating densities should be optimized for specific cell lines.

### Neural differentiation of mouse NS and human NS cell lines

Differentiation of both mouse NS cells and human NS cells is induced by the sequential withdrawal of EGF and FGF-2<sup>1,2,3</sup>. Medium should be changed every 2 - 3 days and cell plating densities should be optimized for specific cell lines.

### Neural differentiation of mouse ES cells in monolayer culture

1. Plate feeder independent early passage ES cells in RHB-A medium onto gelatin-coated tissue culture plastic at  $0.5 - 2 \times 10^4$  cells/cm<sup>2</sup>.
2. Change medium every 1 - 2 days. Considerable ES cell death concomitant with early neural differentiation is to be expected.
3. Monitor for neuronal differentiation by cellular morphology and staining for neuronal markers.

Neural differentiation should be apparent after 4 - 6 days and neuronal maturation should occur after 7 - 9 days. The above protocol is recommended as a starting protocol. Specific culture conditions should be established for individual cell lines.