PRODUCT INFORMATION

RHB-A®

Catalog Number: Y40001

Size: 500 ml

Applications: Demonstrated applications of RHB-A include:

- Maintenance and expansion of adherent, mouse and human neural stem (NS) cells
- Neural differentiation of mouse embryonic stem (ES) cells in monolayer culture
- Derivation of NS cells from mouse 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[1 - 4, 8] 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[1]. RHB-A in the presence of EGF and FGF-2 also supports the derivation of clonogenic NS cell lines[1].

RHB-A is significantly more effective than NDiff® 227 (Cat. #Y40002) at promoting the improved differentiation of mouse embryonic stem cells into neurons without the need for growth factor supplementation[6, 7].

Culture of adherent NS cells in RHB-A with sequential growth factor withdrawal leads to differentiation into functional neurons[1].

RHB-A supplemented with EGF and FGF-2 has recently been used for the propagation of glioblastoma stem cell lines[5].

In vitro human brain organotypic primary cell cultures with expansion and differentiation of radial glia derived NS cells[8].

Storage: Upon receipt, store at -20°C until ready to use. When stored under these conditions, the product remains stable until the expiration date specified on the product label.

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

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

Preparation:

Thaw the medium in a water bath (37℃) in the dark, and remove it from the water bath just before the medium has completely thawed (i.e., do not allow the media to warm up). Then, mix the medium gently and thaw completely. Alternatively, thaw the medium at 4℃ while protecting from light. If a precipitate appears in the medium, leave it at 4℃ overnight to completely dissolve the precipitate. Do not use media with visible precipitate; ensure it is dissolved before use.

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 Data:

Please see the Certificate of Analysis (CoA) for each lot. You can download the CoA on Takara Bio website.

References:

1. Sun Y, Pollard S, Conti L, Toselli M, Biella G, Parkin G, Willatt L, Falk A, Cattaneo E, and Smith A. Long-term tripotent differentiation capacity of human neural stem (NS) cells in adherent culture. *Molecular and Cellular Neuroscience*. (2008) **38**: 245-258.
2. Conti L, Pollard SM, Gorba T, Reitano E, Toselli M, Biella G, Sun Y, Sanzone S, Ying QL, Cattaneo E, and Smith A. Niche-Independent symmetrical self-renewal of a mammalian tissue stem cell. *PLoS Biology*. (2005) **3**(9): e283.
3. Pollard SM, Conti L, Sun Y, Goffredo D, and Smith A. Adherent Neural Stem (NS) cells from fetal and adult forebrain. *Cerebral Cortex*. (2006) **16**: 112-120.
4. Pollard SM, Wallbank R, Tomlinson S, Grotewold L, and Smith A. Fibroblast growth factor induces a neural stem cell phenotype in foetal forebrain progenitors and during embryonic stem cell differentiation. *Molecular and Cellular Neuroscience*. (2008) **38**: 393-403.
5. Pollard SM, Yoshikawi K, Clarke ID, Danovi D, Stricker S, Russell R, Bayani J, Head R, Lee M, Bernstein M, Squire J, Smith A, and Dirks P. Glioma stem cell lines expanded in adherent culture have tumor-specific phenotypes and are suitable for chemical and genetic screens. *Cell Stem Cell*. (2009) **4**: 568-580.
6. Diogo MM, Henrique D, and Cabral JM. Optimization and integration of expansion and neural commitment of mouse embryonic stem cells. *Biotechnology and Applied Biochemistry*. (2008) **49**: 105-112.
7. Abranches E, Silva M, Pradier L, Schulz H, Hummel O, Henrique D, and Bekman E. Neural differentiation of embryonic stem cells *in vitro*: A road map to neurogenesis in the embryo. *PLoS ONE*. (2009) **4**(7): e6286.
8. Hansen DV, Lui JH, Parker PRL, and Kriegstein AR. Neurogenic radial glia in the outer subventricular zone of human neocortex. *Nature*. (2010) **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[1, 4] (NOTE: The recommended concentrations of EGF & FGF-2 are 10 - 20 ng/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.

For culturing human NS cell lines, use tissue culture plasticware precoated with 10 μg/ml laminin solution[1].

For culturing mouse NS cell lines, use tissue culture plasticware which has been precoated with poly-ornithine/laminin by treating it with 0.01% poly-L-ornithine hydrobromide for 30 - 60 minutes, washing twice with PBS, then treating it with 10 μg/ml laminin solution for at least 2 hours.[4]

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[1, 2, 3]. 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 x 10^4 cells/cm^2.
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.

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Note
This product is for research use only. It is not intended for use in therapeutic or diagnostic procedures for humans or animals. Also, do not use this product as food, cosmetic, or household item, etc.
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