PRODUCT INFORMATION

NDiff® 227

Catalog Number: Y40002

Size: 500 ml

Applications: Demonstrated applications of NDiff 227 include:

- Neural differentiation of mouse embryonic stem (ES) cells in monolayer culture
- Mouse ES cell culture when used with appropriate supplementation

Description: NDiff 227 is a proprietary, defined, serum-free medium for the neural differentiation of mouse ES cells in adherent monolayer culture conditions as described in Ying QL, *et al*. (2003)[1].

NDiff 227 has also been shown to support the serum-free, feeder-free culture of mouse ES cells when supplemented as described in Ying QL, *et al.* (2003)[2].

More recently, 227-like medium supplemented with growth factors has also been used to successfully maintain feeder-free human ES cells in culture[3, 4].

When supplemented with FGF, serum-free 227 also generates Anterior Definitive Endoderm (ADE) precursors from mouse ES cells, which can then be differentiated into liver and pancreas with enhanced efficiency[5].

Storage: Upon receipt, store at -20℃ 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℃ 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 the medium from the water bath just before it has completely thawed (i.e., do not allow the medium to warm up). Then, mix gently and thaw completely. Alternatively, thaw the medium at 4℃ while protecting from light. If a precipitate appears, leave the medium at 4℃ overnight to completely dissolve the precipitate. Do not use medium with visible precipitate; ensure it is dissolved before use.

NDiff 227 is a complete, ready-to-use medium for the neural differentiation of mouse ES cells (see 'Recommended Use' below). Refer to the appropriate References to use as a mouse ES cell culture medium[2], to maintain feeder-free human ES cells in culture[3, 4], or to derive ADE from mouse ES cells[5].

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

Takara Bio Inc. **https://www.takara-bio.co.jp**
Takara Bio USA, Inc. **https://www.takarabio.com**

References:

1. Ying QL, Stavridis M, Griffiths D, Li M, and Smith A. Conversion of embryonic stem cells into neuroectodermal precursors in adherent monoculture. *Nature Biotechnology*. (2003) **21**: 183-186.

2. Ying QL, Nichols J, Chambers I, and Smith A. BMP induction of Id proteins suppresses differentiation and sustains embryonic stem cell self-renewal in collaboration with STAT3. *Cell*. (2003) **115**: 281-292.

3. Li Y, *et al.* Expansion of Human Embryonic Stem Cells in Defined Serum-Free Medium Devoid of Animal-Derived Products. *Biotechnology and Bioengineering*. (2005) **91**: 688-698.

4. Yao S, *et al.* Long-term self-renewal and directed differentiation of human embryonic stem cells in chemically defined conditions. *PNAS*. (2006) **103**(18): 6907-6912.

5. Morrison G, Oikonomopoulou I, Portero Migueles R, Soneji S, Livigni A, Enver T, and Brickman J. Anterior Definitive Endoderm from ESCs reveals a role for FGF signaling. *Cell Stem Cell*. (2008) **3**: 402-415.

Recommended Use: **Neural differentiation of mouse ES cells in monolayer culture**

1. Plate feeder independent early passage ES cells in NDiff 227 medium onto gelatin-coated tissue culture plastic at 2.5 - 10 x 10^3 cells/cm^2.
2. Change medium every 1 - 2 days. 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.