

## Neural Differentiation Medium

Neural Differentiation complete medium is formulated to differentiate human or neural stem cells (NSCs) into a pan-neural population of mature neurons and support cells. Neural Differentiation medium contains glutamine but no antibiotics. After all the components are added to the basal medium and filtered to ensure sterility, the media is complete and ready to use to differentiate NSCs into many sub-types of neurons, astrocytes and oligodendrocytes. NSCs differentiate best when cultured in a humidified incubator set at 6% CO<sub>2</sub>, 37°C and oxygen concentrations below 5% O<sub>2</sub>.

### Making Neural Differentiation Complete Medium

1. Neural Differentiation Medium (Cat.# 21004-250) includes the following components:
  - a. Neural Differentiation Basal Medium
  - b. Neural Differentiation Supplement Pack includes the following components:

Supplement Pack Components
Neural Differentiation Base Supplement (contains glutamine)
Laminin
BDNF
GDNF

### Preparing Neural Differentiation Medium

1. Warm Neural Differentiation basal medium to room temperature
2. Thaw Neural Differentiation Supplement and growth factors
3. Aseptically add supplements to basal medium in order
  - a. Neural Differentiation Base Supplement - transfer entire contents of the vial then rinse the vial with Neural Differentiation basal medium
  - b. Laminin and growth factors BDNF and GDNF
4. Antibiotics are optional but may be added. The cells have been tested in media containing 150ul of Gentamicin (Gibco Cat. # 15750-060 50mg/ml) in 250ml media.
5. Filter through 0.22µm filter unit
6. Store Neural Differentiation complete medium at 4°C in the dark until ready for use
7. Warm Neural Differentiation complete medium to room temperature immediately before use.
  - a. Do not leave the Neural Differentiation complete medium in a water-bath for longer than 5 minutes as this may result in degradation of the growth factors and glutamine.
8. Use Neural Differentiation complete medium within two weeks

**Use Restrictions:** The Products and components of the Products are to be used for RESEARCH PURCHASES ONLY and are not for use in humans or animals. No rights expressed or implied are conveyed by the sale of the Products to make, have made, offer to sell or sell the Products alone or in combination with any other products or to use for commercial purposes without Vesta Biotherapeutics' prior written consent.