



## STEMEZ™ hN2 Discovery Kit

### Kit Contents

- 1 vials STEMEZ™ hN2 Human Neural Cells
- 1 bottles of AB2™ Basal Neural Medium
- 1 vials of ANS™ Supplement

### Required but not Supplied

- LIF (10 µg/ml)
- L-Glutamine (200 mM)

### Optional but not Supplied

- Pen/Strep (100X)

### Unpacking and Storage Instructions

#### STEMEZ™ hN2 Human Neural Cells

- Cells must be moved from dry ice to liquid nitrogen IMMEDIATELY. Temperature fluctuations will have adverse effects on cell health and viability.
- When stored in the recommended storage conditions (liquid nitrogen), **STEMEZ™ hN2 Human Neural Cells** are stable up to 18 months.

#### AB2™ Neural Medium and Supplements

- Upon arrival, store AB2™ Medium at 2-8°C protected from light.
- Upon arrival, store ANS™ Supplement at -20°C.
- After supplements are thawed, use within one month.
- Do not refreeze

### Supplementing the AB2™ Basal Medium

1. Decontaminate the external surfaces of all supplement vials and the medium bottle with ethanol or isopropanol.
2. Aseptically open each supplement vial and add the amount indicated below to the basal medium with a pipette.

To each 97 mL bottle of AB2™ Neural Medium, add:	
ANS™ Supplement	2 mL
LIF	100 µL
L-Glutamine	1 mL
Pen/Strep	1 mL

3. Supplemented medium should be stored at 2-8°C, protected from light. The medium should be given an expiration date of 1 month **after thawing of the ANS™ Supplement**, not the supplementation of the medium. Dispense the complete medium into aliquots to avoid repeated heating prior to each use.

## **Plate Coating Protocol**

### **Protocol Description:**

STEMEZ™ hN2 Cells form adherent monolayer cultures when grown on cell culture plates pre-coated with substrate. We recommend pre-coating your plates with Matrigel™ using the following protocol.

<b>Required but not supplied:</b>
BD Matrigel™ Basement Membrane Matrix
Dulbecco's Modified Eagle's Medium
Phosphate Buffered Saline with Ca and Mg
Tissue culture treated polystyrene plate

### **To coat dishes perform the following steps:**

1. Thaw BD Matrigel™ at 2-8°C overnight. Matrix will gel rapidly at 22°C to 35°C. Keep Matrigel™ on ice and use pre-cooled pipettes, plates and tubes when preparing. Gelled Matrigel™ may be re-liquified if placed at 2-8°C on ice for 24 to 48 hours.
2. Handle using aseptic technique in a laminar flow hood.
3. Once BD Matrigel™ Matrix is thawed, swirl vial to be sure that material is evenly dispersed.
4. Place thawed vial of BD Matrigel™ Matrix in sterile area, decontaminate the external surfaces with ethanol or isopropanol and air dry. BD Matrigel™ Matrix may be gently pipetted using a pre-cooled pipette to ensure homogeneity.
5. Dilute Matrigel™ 1:200 with cooled Dulbecco's Modified Eagle's Medium. Keep on ice.
6. Using the chart below, add the corresponding volume of diluted Matrigel™ to the plate size being used. Swirl to ensure the entire surface of the plate or flask is covered with the Matrigel™ solution.
7. Place dishes at 2-8°C for 1-3 hours.
8. Rinse thoroughly with PBS.
9. Remove PBS and either use immediately or allow to dry for later use. Dried plates can be stored at 2-8°C for up to 2 weeks. See "Rehydration of Pre-Coated Dishes" below.

### **Recommended volumes to coat flasks:**

<b>Plate/Flask</b>	<b>Working Volume</b>
96 well plate	100 µl/well
35 mm dish	2 mL
6 well plate	2 mL/well

### **Rehydration of Pre-Coated Dishes**

Before use, rehydrate the pre-coated dishes by adding PBS (use volumes recommended above). Warm the plate in a humidified incubator at 37°C for 30 minutes prior to use. Aspirate the PBS before using for cell culture.

**NOTE: IF USING CELLS IN A FORMAT OTHER THAN DESCRIBED ABOVE PLEASE CONTACT FOR TECHNICAL ASSISTANCE.**

## **Cell Thawing Protocol**

### **Protocol Description:**

STEMEZ™ hN2 Human Neural Cells form adherent monolayer cultures when grown on cell culture plates pre-coated with substrate. We recommend thawing your STEMEZ™ hN2 Cells using the following protocol.

#### **Required but not supplied:**

Pre-coated plates  
(see hN2 plate coating protocol)

### **To Plate the cells perform the following steps:**

1. Do not thaw the cells until the recommended medium and appropriately coated plasticware and/or glassware are on hand.
2. Remove the vial from liquid nitrogen and incubate in a 37°C water bath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells.

*IMPORTANT: Do not vortex the cells. Breaking cells down to single cell suspensions will significantly increase cell death.*

3. As soon as the cells are completely thawed, disinfect the outside of the vial with ethanol or isopropanol. Proceed immediately to the next step.
4. In a laminar flow hood, use a 1 or 2 mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful to not introduce any bubbles during the transfer process.
5. Using a 10 mL pipette, slowly add dropwise 9 mL of supplemented AB2™ Neural Medium (pre-warmed to 37°C) to the 15 mL conical tube.

*IMPORTANT: Do not add the whole volume of medium at once to the cells. This may result in decreased cell viability due to osmotic shock.*

6. Gently mix the cell suspension by slow pipetting up and down twice. Be careful to not introduce any bubbles.

*IMPORTANT: Do not vortex the cells. Breaking cells down to single cell suspensions will significantly increase cell death.*

7. Centrifuge the tube at room temperature at 140 x g for 3-5 minutes to pellet the cells. NOTE: hN2 cells are sensitive to higher centrifugation speeds.
8. Aspirate as much of the supernatant as possible. Steps 4-8 are necessary to remove residual cryopreservative (DMSO).
9. Resuspend the cells in supplemented AB2™ Neural Medium (pre-warmed to 37°C).
10. Plate cells onto a pre-coated plate and fill with supplemented AB2™ Neural Medium to the appropriate volume using the chart below.
11. Incubate the cells at 37°C in a 5% CO<sub>2</sub> humidified incubator.
12. Exchange the medium with fresh supplemented AB2™ Neural Medium 24 hours post plating. Exchange with fresh medium every 3-4 days thereafter. Use caution not to dislodge the cells; do not pipette media directly onto the cells but rather onto the side of the culture dish.

### **Recommended plating density:**

<b>Format</b>	<b>Plating per Vial</b>	<b>Working Volume</b>
96 well plate	24 wells	200 µl/well
35 mm dish	1 dish	2 mL
6 well plate	1 well	2 mL/well

**NOTE: IF USING CELLS IN A FORMAT OTHER THAN DESCRIBED ABOVE PLEASE CONTACT FOR TECHNICAL ASSISTANCE.**

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