



STEMEZ™ hNP1 Expansion Kit

Kit Contents

- 1 vial of STEMEZ™ hNP1 Human Neural Progenitor Cells
- 1 bottle of AB2™ Neural Medium
- 5 vials of ANS™ Supplement

Required but not Supplied

- bFGF (50 µg/ml)
- L-Glutamine (200mM)

Optional but not Supplied

- Pen-Strep (100X)

Unpacking and Storage Instructions

STEMEZ™ hNP Human Neural Progenitor 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), hNP1™ Human Neural Progenitor Cells are stable up to 18 months.

Medium and Supplements

- Upon arrival, store the AB2™ Neural 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 make 100 ml of complete medium:	
AB2™ Neural Medium	96 ml
ANS™ Supplement	2 mL
bFGF	40 µl
L-Glutamine	1 mL
Pen/Strep (optional)	1 mL

3. Supplemented medium should be stored at 2-8°C, protected from light. The complete medium should be given a 2 week expiration date. Dispense the complete medium into aliquots to avoid repeated heating prior to each use.

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Plate Coating Protocol

Protocol Description:

STEMEZ™ hNP1 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.

Required but not supplied:
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:

Plate/Flask	Working Volume
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.

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Cell Thawing Protocol

Protocol Description:

STEMEZ™ hNP1 Human Neural Progenitor Cells form adherent monolayer cultures when grown on cell culture plates pre-coated with substrate. We recommend thawing your STEMEZ™ hNP Human Neural Progenitor Cells using the following protocol.

Required but not supplied:

Pre-coated plates
(see 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 fully 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.

1. Centrifuge the tube at room temperature at 200 x g for 4 minutes to pellet the cells.
2. Aspirate as much of the supernatant as possible. Steps 4-8 are necessary to remove residual cryopreservative (DMSO).
3. Resuspend the cells in a total volume of 2 mls of fully supplemented AB2™ Neural Medium (pre-warmed to 37°C).
4. We recommend plating a vial of hNP1™ cells onto a 35 mm dish with a total of 2 mL of media.
5. Incubate the cells at 37°C in a 5% CO₂ humidified incubator.
6. Exchange the medium with fresh fully supplemented AB2™ Neural Medium 24 hours post plating. Exchange with fresh medium every other day 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.
7. Once the hNP1™ cells reach 90% - 100% confluence, they can be dissociated manually and passaged. The cells should be maintained at a high density at all times and thus a recommended passaging ratio of 1:2 – 1:3.

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Subculture of STEMEZ™ hNP1 Cells

1. Once the STEMEZ™ hNP1 Cells reach 90-100% confluence, carefully remove the medium from the 35 mm dish.
2. Apply 2 mL fully supplemented AB2™ Neural Medium (pre-warmed to 37°C) to the cells so that the cells can be harvested in fresh medium.
3. Using a pipette, manually detach the cells from the dish by slow pipeting up and down the dish. Be careful to avoid introducing any bubbles. We recommend using a 200 µl or 1000 µl manual pipette to dislodge the attached cells. Alternatively, cells can be dislodged with a sterile cell scraper.
4. Plates should be observed to ensure that all cells have been removed. This is most easily accomplished by working under a dissection microscope within a laminar flow hood, but can also be achieved by frequent observation under a bright field or phase contrast microscope.
5. Transfer the dissociated cells to a 15 mL conical tube. Inspect the plate to ensure that all the cells have been removed.
6. If necessary, cells can be centrifuged at 200 x g for 4 minutes in order to count the cells and replate at a specific concentration.
7. Plate the cells to the desired density into the appropriately coated flasks, plates or wells (see plate coating protocol) in fully supplemented AB2™ Neural Medium. We recommend keeping the cells at a high cell density by passaging 1:2 – 1:3.
8. Incubate the cells at 37°C in a 5% CO₂ humidified incubator.
9. Exchange the medium with fresh fully supplemented AB2™ Neural Medium 24 hours post plating. Exchange with fresh

medium every other day 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.

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