NeuroNet™ Neural Discovery Kit (Cat#NN60001)

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Kit Contents

- 1 vial NeuroNet™Pure Human Neurons (Part # 7014)
- 1 x 500ml bottle of AB2™ Basal Neural Medium (Part # 7011.3)
- 1 x 5ml vial of ANS™ Neural Medium Supplement (Part # 7011.4)

Required but not Supplied

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

Optional but not Supplied

- Penicillin (5,000 U/ml)/Streptomycin (5,000 µg/ml)

Unpacking and Storage Instructions

NeuroNet™ Human Neurons

- **Cells must be moved from dry ice to liquid nitrogen IMMEDIATELY.** Temperature fluctuations will have adverse effects on cell health and viability.

AB2™ Basal Neural Medium and ANS Neural Medium Supplement

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

US Patent 6,200,806
US Patent 8,178,089

Supplementing the AB2™ Basal Neural 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.

For 100mls of AB2™ Neural Medium, add:	
AB2™ Basal Neural Medium	97mls
ANS™ Neural Medium Supplement	2 mL
LIF (10 µg/ml)	100 µL
L-Glutamine	1 mL
Penicillin/Streptomycin (optional)	1 mL

3. Filter the supplemented medium through a sterile 0.22µm filter prior to use.

4. Fully supplemented medium should be stored at 2-8°C, protected from light. The medium should be given an expiration date of 2 weeks after supplementation. Note: Any remaining unused thawed ANS™ Neural Medium Supplement must be used within 1 month. Dispense the complete medium into aliquots to avoid repeated heating prior to each use.

Plate Coating Protocol

Protocol Description:

NeuroNet™ cells form adherent monolayer cultures when plated on cell culture plates precoated with substrate. We recommend precoating your plates with Matrigel™ using the following protocol.

Required but not supplied:
BD Matrigel™ Basement Membrane Matrix
Dulbecco's Modified Eagle's Medium
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.** Note: Gelled Matrigel™ may be re-liquefied 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. Note: For long term storage, Matrigel™ can be diluted 1:2 with cooled Dulbecco's Modified Eagle's Medium and refrozen into aliquots. These aliquots can then be thawed as describe in Step 1 above and stored at 4°C for up to 1 week before diluting further

US Patent 6,200,806
US Patent 8,178,089

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. Allow dishes to incubate at room temperature for at least 15 minutes, but no longer than 1 hour before use.

8. Remove Matrigel™ and use plates immediately. Freshly prepared Matrigel plates should always be used with NeuroNet™ Human Neural Cells. To avoid waste, the 1:200 dilution of Matrigel™ can be stored at 4°C for up to 1 week to coat fresh plates as needed.

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

NOTE: IF USING CELLS IN A FORMAT OTHER THAN DESCRIBED ABOVE PLEASE CONTACT US FOR TECHNICAL ASSISTANCE AT 706-542-9857 OR TECHSUPPORT@ARUNABIOMEDICAL.COM.

Cell Thawing Protocol

Protocol Description:

NeuroNet™ Human Neural Cells form adherent monolayer cultures when grown on cell culture plates pre-coated with substrate. Thawing your NeuroNet™ Cells using the following protocol is critical for success.

Required but not supplied:
Pre-coated plates (see NeuroNet™ 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 using hemostats, **gently swirl** the vial and incubate in a 37˚C water bath. Closely monitor until the cells are thawed to a point where a trace amount of ice crystal remains on top. The remaining ice should disappear quickly upon extraction from the water bath incubator. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells.

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.

US Patent 6,200,806
US Patent 8,178,089

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 5 minutes to pellet the cells. NOTE: NeuroNet™ cells are sensitive to higher centrifugation speeds.

Important: Please refer to your centrifuge manual to determine appropriate and corresponding settings. The proper G-force is critical.

8. Aspirate as much of the supernatant as possible.
   Note: Steps 4-8 are necessary to remove residual cryopreservative (DMSO).
9. Resuspend the cells in 1mL supplemented AB2™ Neural Medium for each NeuroNet™ vial thawed (pre-warmed to 37°C) to the desired cell density.
10. Plate cells onto a pre-coated plate and fill with supplemented AB2™ Neural Medium to the appropriate volume using the chart below.
11. Place the 35mm dish of plated cells on a shelf of a 37°C 5% CO2 humidified incubator then gently move the plate in a side-to-side and back-and-forth manner to disperse cells in a uniform manner.
12. Incubate the cells at 37°C in a 5% CO2 humidified incubator.
13. 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: (~100,000 cells/cm^2) The plating density should be adjusted per assay requirements.

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

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US Patent 6,200,806
US Patent 8,178,089

Recommended but not supplied components

Component	Vendor	Cat# Number
Matrigel	BD	354234 or 354277
DMEM	Sigma	D6046
L-glutamine	Invitrogen	25030
Penicillin/Streptomycin	Invitrogen	15070
LIF	Millipore	LIF1010
6-well plates	BD Falcon	353046
35mm plates	BD Falcon	353001
96 well plates	Corning	3603

US Patent 6,200,806
US Patent 8,178,089