Mouse HB9-GFP Motor Neuron Cells-(Cat# 7025)

FOR RESEARCH USE ONLY

Kit Contents

1 vial 750,000 HB9-GFP Motor Neuron Cells

Unpacking and Storage Instructions

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

Mouse Motor Neuron Thawing & Plating Protocol

One vial of Motor Neurons should be thawed into one T25 TPP tissue culture dishes. Prior to thaw, the tissue culture dish will need to be precoated with Matrigel™ using the following protocol. We use BD Cat# 356234.

Plate Coating Protocol

1. Thaw BD Matrigel™ at 2-8ºC overnight. Matrix will gel rapidly at 22°C to 35°C. Keep Matrigel™ on ice and use precooled pipettes, plates and tubes when preparing. Gelled Matrigel™ may be reliquified if placed at 2-8ºC 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. For long term storage, Matrige^{lTM} 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 and stored at 4°C for up to 1 week before diluting further.
5. Dilute Matrigel™ 1:200 with cooled Dulbecco's Modified Eagle's Medium or other basal 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^{TM} plates should always be used with ArunA's mouse Motor Neurons. It is recommended that some amount of culture media be added to the plates immediately so that the plates do not dry out. To avoid waste, the 1:200 dilution of Matrigel^{TM} can be stored at 4°C for up to 1 week to coat fresh plates as needed.

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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
T25 flask	4 mL

Motor Neuron Culture Medium: (stock concentrations are what we use in house and may need to be modified from original stocks)

Component	Stock concentration	Final concentration	For 50 mL
Advanced DMEM/F12 (Life Technologies 12634-010)			25 mL
AB2™ Basal Neural Medium (Neuromics AB27011.3)			25 mL (500 ml included)
Knockout Serum Replacement (Life Technolgies 10828010)			5 mL
L-Glutamine (Life Technologies 25030-081)	100 X	1 X	0.5 mL
Pen/Strep (Life Technologies 15070-063)	100 X	1 X	0.5 mL
B-mercaptoethanol (Sigma M7522)	1 M (diluted in PBS -/- , stored @ -20 C)	0.1 mM	5 µL
GDNF (Neuromics PR27022)	100 µg/mL in H_2O	10 ng/mL	5 µL Purchase options: 2 ug:=108 USD/ 10 ug= 205 USD
CTNF (Sigma C3710)	100 µg/mL in PBS w/ 0.1% BSA	10 ng/mL	5 µL

Cell Thawing Protocol

Note: It is recommended that cells are not centrifuged post-thaw, as centrifugation greatly decreases the number of cells recovered; however, centrifugation may be necessary for applications requiring high cell density. See alternative protocol below if high cell density is need.

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 37oC 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 70% 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 Motor Neuron Culture Media (prewarmed 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.
7. Centrifuge the tube at room temperature at 200 x g for 4 minutes to pellet the cells.

NOTE: These cells do not always form a pellet after centrifugation. Leave ~ 10 uL of cell solution during aspiration.

8. Aspirate off the supernatant, leaving approximately 10 uL.
9. Resuspend the cells in Motor Neuron Culture Media (pre-warmed to 37°C) to the desired cell density. We plate these cells @ ~ 40,000 cells/cm^2 (1 vial per T25 flask).
10. Plate cells onto a pre-coated plate and fill with Motor Neuron Culture Media to the appropriate volume using the chart below. Gently move the plate in a sideto-side and back-and-forth manner to disperse cells in a uniform manner.
11. Incubate the cells at 37°C in a 5% CO2 humidified incubator.
12. Exchange the medium with fresh Motor Neuron Culture Media 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: (~40,000 cells/cm^2) The plating density should be adjusted per assay requirements.

Format	Plating per Vial	Working Media Volume
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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Cell Thaw Protocol (with centrifugation)

Note: As stated above, it is recommended that cells are not centrifuged post-thaw, as centrifugation greatly decreases the number of cells recovered. It is not uncommon to lose ~50% of the cells during centrifugation. Centrifugation is only recommended for applications requiring high cell density.

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 37oC 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 70% 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 Motor Neuron Culture Media (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.
7. Centrifuge the tube at room temperature at 1000rpm for 10 minutes to pellet the cells.

NOTE: These cells do not always form a pellet after centrifugation. Leave ~ 10 uL of cell solution during aspiration.

8. Aspirate off the supernatant, leaving approximately 10 uL.
9. Resuspend the cells in Motor Neuron Culture Media (pre-warmed to 37°C) to the desired cell density. ArunA plates these cells @ ~ 40,000 cells/cm2 (1 vial per T25 flask).
10. Plate the entire 10mL cell suspension onto a pre-coated plate (~40,000 cells/cm2 or 1 vial per T25 flask). Gently move the plate in a side-to-side and back-and-forth manner to disperse cells in a uniform manner.
11. Incubate the cells at 37°C in a 5% CO2 humidified incubator.
12. Exchange the medium with fresh Motor Neuron Culture Media 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.

Cell Culture Protocol

1. Mouse Motor Neurons require media changes twice a week with Motor Neuron Culture Medium. These cells are lightly adherent so it is recommended that some old media be left on the plates during media changes.

IMPORTANT: The first media change (24 hours post-plating) should be full media change to remove any residual freezing media. Subsequent media changes can be partial to avoid detaching cells.

2. To change the media, aspirate most of the existing media and gently add prewarmed Motor Neuron Culture Medium to the cells. Continue to incubate @ 37°C, 5% CO_2.

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

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