Adult Hippocampal Arctic Ground Squirrel Neural Stem Cells (AGS-NSC)

CATALOG NUMBER: SCCE002

LOT NUMBER:

QUANTITY: 1 ampoule containing 500,000 cells. Guaranteed minimum 60% viability when thawed from cryopreservation

BACKGROUND: Adult Hippocampal Arctic Ground Squirrel Neural Stem Cells (AGS-NSC) are isolated from the hippocampus of adult Arctic Ground Squirrels following hibernation. Both the isolated cells and the whole animals are tolerant of ischemic insult and reperfusion. Using these cells, researchers can identify potentially novel proteins and genes key to neuronal tolerance, neuroprotection and neurogenesis. Millipore's AGS-NSC can be used to screen drugs, genes and proteins for protective changes caused by oxygen and/or glucose deprivation. These cells can potentially identify future targets for novel stroke therapeutics. As stem cells, these cells can be used in transplantation studies using rodent stroke models to investigate tolerance to ischemic injury.

This product is for Research Use Only. This product is not approved for human or veterinary use, or for use in *in vitro* diagnostic or clinical procedures.

STORAGE: AGS-NSC's are sold as cryopreserved ampoules. Ampoules are shipped in insulated packages containing dry ice to insure the cells remain in a cryopreserved state. To maintain the integrity of the cells, unpack the products immediately upon receipt. The cryopreserved cells should be placed in the liquid or gas phase of a liquid nitrogen dewar for storage. If the cells are to be thawed and plated within 24 hours, they may be stored at -80°C.

Do not store the ampoule for more than 24 hours at -80°C as the cells will slowly degrade at this temperature. Long-term storage of the ampoule must be in a liquid nitrogen dewar or a mechanical freezer designed for cryopreserved cell storage that maintains a temperature below -135°C.

Millipore recommends storing the cryopreserved vials in liquid nitrogen vapor phase. Handle cryopreserved vials with caution. Always wear eye protection and gloves when working with cell cultures. Aseptically vent any nitrogen from cryopreserved vials in a biosafety cabinet prior to thawing the vials in a water bath. If vials must be stored in liquid phase, the vials should be transferred to vapor phase storage or -80°C for at least 24 hours prior to being thawed.

QUALITY CONTROL: AGS-NSC are tested for normal NSC morphology, and tested negative for mycoplasma, bacterial and fungal growth. They maintain normal morphology in expansion medium (SCMA002) and differentiate into neurons in AGS-NSC differentiation medium (SCMA003).

MEDIA PREPARATION: AGS-NSC should be cultured in AGS-NSC Expansion medium (warmed to 37°C) for best results. Please see specific media data sheets for further information (SCMA002 and SCMA003).

The media and reagents that are to be prepared from the supplied kit components are shown below. It is recommended that the media be prepared fresh on a weekly basis.

Medium/Reagent Preparation	Components and Volumes	
AGS-NSC Expansion Media	AGS-NSC Basal Medium (SCMA-BM) B-27™ (Invitrogen)	49 mL 1.0 mL
AGS-NSC Basal Medium with 5% FBS (for use as a neutralizing solution)	AGS-NSC Basal Medium (SCMA-BM) FBS	95 mL 5 mL
Arctic Ground Squirrel NSC Differentiation Media Kit	AGS-NSC Basal Medium (SCMA-BM) ITS-*p*	49.5 mL 0.5 mL

POLY-L-ORNITHINE COATING OF FLASKS:

1. Thaw Poly-L-Ornithine (10 µg/mL) from AGS-NSC Expansion Kit (Solution can be stored between 2-8°C for up to one month once thawed).
2. Add 10 mL Poly-L-Ornithine working solution to one (1) T-75 flask and evenly distribute the solution over the entire culture surface of the flask. (Adjust amount of working solution for other culture vessels that may be used. Use 0.5 – 1.0 mL Poly-L-Ornithine working solution per 10 cm^2 culture surface.)
3. Incubate flasks at 37°C for a minimum of 24 hours. (Use of an incubator without added CO_2 is preferred for incubation of Poly-L-Ornithine coated culture vessels.) Culture vessels should be used within five days of adding Poly-L-Ornithine working solution.
4. Prior to use, aspirate Poly-L-Ornithine working solution from the flask and wash twice with sterile tissue culture grade water. Aspirate liquid to near dry before use

PRE-WARMING MEDIUM

- Repeated warming of the entire bottle over extended periods may cause degradation of the medium and reduced shelf life.
- If only using a small volume of medium (less than 50 mL), it is recommended to warm only the volume needed in a sterile conical tube.
- If warming the entire bottle, the medium temperature may be checked using the special thermometer attached to the side of the bottle. Medium will take from 10 to 30 minutes to warm to 37°C depending on the volume. Do not leave medium in water bath for extended periods.

THAWING AND PLATING CRYOPRESERVED CELLS:

1. Prepare AGS-NSC Basal Medium with 5% FBS by adding 5 mL FBS (provided in kit) to 95 mL AGS-NSC Basal Medium.
2. Prepare AGS-NSC Expansion Medium by adding 2 mL B-27™ Supplement to 100 mL AGS-NSC Basal Medium.
3. Warm 25 mL of AGS-NSC Basal Medium with 5% FBS and 20 mL of AGS-NSC Expansion Medium in a 37°C water bath.
4. Remove one ampoule of cryopreserved cells from liquid nitrogen storage dewar and check cap to be sure ampoule is securely sealed.
5. Hold ampoule in a 37°C water bath so that only the bottom half of the ampoule is in the water. To avoid potential contamination, do not allow the ampoule cap to make contact with the water. This procedure should take

approximately one minute or until ampoule is almost completely thawed—a small piece of ice should still be visible—do not over thaw as this may damage the cells.

6. Place ampoule in a biological safety cabinet and sterilize the exterior of the ampoule using 70% EtOH.
7. Carefully remove the cap, avoiding contamination or spatter.
8. Gently re-suspend the cells in the ampoule using a 1 or 2 mL sterile pipette.
9. Transfer the contents of the vial to 25 mL of warm AGS-NSC Basal Medium with 5% FBS.
10. Centrifuge the cells at 150 x g for 5 minutes. For best results adjust speed and time to suit your individual centrifuge.
11. Aspirate the supernatant fluid; take care to not aspirate the pellet.
12. Resuspend the cells in 20 mL of AGS-NSC Expansion Medium.
13. Transfer the resuspended cells to one (1) T-75 flask.
14. Add 40 µL of rh FGF-basic to the flask.
15. Gently rock the culture vessel side-to-side to evenly distribute cells within the vessel.
16. Place seeded culture vessel in the incubator at 37°C, 5% CO_2.

EXPANSION OF AGS-NSC:

1. Two days (~48 hours) after thawing and plating the cells, a full medium replacement should be performed using AGS-NSC Expansion Medium plus a 40 µL spike of rh FGF-basic.
2. The following day, spike with 40 µL of rh FGF-basic.
3. Continue daily spikes with rh FGF-basic and full medium changes every other day until the flask is near 70%–80% confluent, approximately 3–5 days.

   Note: The rh FGF-basic can be stored at 4°C for up to one week and maintain activity. The rh FGF-basic can also go through one additional freeze/thaw if more time is needed.

TRYPSINIZATION OF CELLS:

1. AGS-NSC may be passaged once the culture is near 70%-80% percent confluent and actively proliferating.
2. Prepare a Poly-L-Ornithine coated flask 24 hours before passaging if the AGS-NSC will be expanded again.
3. Aspirate the medium from the culture vessel.
4. Add 2.5 mL of 0.05% Trypsin/0.02% EDTA to the vessel for each 75 cm^2 of surface area. Swirl gently to ensure all cells are coated with the Trypsin/EDTA. This normally takes from 30-60 seconds depending on the confluence of the cells. Do not over trypsinize as this will damage the cells.
5. Detach the cells by gently tapping the culture vessel from several sides.
6. Once the cells become detached, add 10 mL of AGS-NSC Basal Medium with 5% FBS to the flask. Gently swirl to ensure all of the trypsin solution is neutralized.
7. Using sterile laboratory techniques, pipette the cells into a sterile centrifuge tube.

   Note: All steps must be completed under sterile conditions in a biological safety cabinet.
8. Centrifuge cells at 150 x g for 5 minutes. For best results adjust speed and time to suit your individual centrifuge type. Do not over centrifuge cells as this will cause cell damage.
9. After centrifugation, the cells should form a clean loose pellet. Aspirate neutralized trypsin from the centrifuge tube and re-suspend the cell pellet in 5-10 mL (per T-75 flask) of pre-warmed AGS-NSC Basal Medium with 5% FBS by gently pipetting up and down with a 2 or 5 mL pipette.
10. Perform a cell count as described below.

STANDARD CALCULATION FOR PLATING OF CELLS:

1. Using sterile technique, transfer 50 µL of the cell suspension to a microcentrifuge tube.

2. Add 350 µL of medium to dilute the concentrated cell suspension and 100 µL of 0.4% Trypan Blue.
3. Mix gently and using a clean hemacytometer, load 10 µL of cell suspension to one or both chambers.
4. Count a minimum of 4 quadrants on the hemacytometer. The blue cells that are positive for Trypan Blue uptake are dead. For accurate cell counts, optimal number of cells per quadrant should be 25-75 cells. After counting the cells, calculate the average of the 4 quadrants. Take the cell count average and multiply by 10,000 and multiply again by the dilution factor (10 if using the recommended volumes) to get the number of cells per mL.

   SAMPLE CALCULATION:

   Total viable cells counted in 4 quadrants = 140
   Total non-viable cells counted in 4 quadrants = 10
   Total cells counted =150, Percent Viability = 140 viable ÷ 150 total x 100 = 93.3%
   Average cells per quadrant = 35
   35 cells/quadrant x 10,000 quadrants/mL x 10 dilution factor = 3,500,000 cells/mL.

INOCULATION OF ANOTHER EXPANSION FLASK:

1. Multiply the desired seeding density (5,300 cells per cm^2 or 400,000 cells per T-75 flask) by the surface area of the vessel(s) to be inoculated. This will give you the total number of cells to inoculate one vessel.
2. Divide the number of cells needed to inoculate one vessel by the number of viable cells/mL in the cell suspension. This will give you the volume of cell suspension with which to inoculate each vessel.
3. Inoculate the cells into the culture vessels prepared with 20 mL of pre-warmed AGS-NSC Basal Medium. Mix gently to evenly distribute the cells and place culture vessels into the incubator at 37°C, 5% CO_2.
4. Add 40 µL of rh FGF-basic to each flask.

   SAMPLE CALCULATION:

   Inoculating 2 T-75 flasks at 400,000 cells/flask:
   5,300 cells/cm^2 x 75 cm^2 = 397,500 (round up to 400,000)
   Calculate volume of cell suspension to inoculate each flask with:
   400,000 cells/flask divided by 3,500,000 cells/mL = 0.114 mL/flask

DILUTION OF CELLS FOR INOCULATION OF WELL PLATES FOR DIFFERENTIATION EXPERIMENTS:

1. Determine volume of cell suspension needed for your experiment by multiplying the number of wells to be inoculated by 0.2 mL per well and add approximately 20% to ensure a large enough volume to inoculate the desired number of wells.
2. Calculate the dilution to 75,000 cells/mL. The cells are currently in AGS-NSC™ Basal Medium with 5% FBS, but the FBS concentration must be decreased to 2%. Determine the volume of FBS in the initial cell suspension. Determine the amount of FBS to be added to the differentiation medium to achieve a 2% final concentration of FBS. Then mix the FBS and Differentiation Medium. Carefully mix the cells with the calculated volume of Differentiation Medium with FBS. See calculations below.
3. Dispense diluted cell suspension to well plates; for example, Poly-L-Lysine coated 96-well plates (200 µL). Incubate the plates for 2 hours at 37°C, 5% CO_2.
4. After the cells have attached, carefully remove 50% (100 µL for 96-well plates) of the medium from each well using large orifice pipet tips.
5. Carefully replace the volume removed with warm AGS-NSC Differentiation Medium, using large orifice pipet tips.
6. Two days after inoculation (~ 48 hours), perform a 50% medium change using AGS-NSC Differentiation Medium.

   SAMPLE CALCULATION:

   1. Using 3,500,000 cells/mL cell suspension
   2. Inoculating 96 wells at 0.2 mL per well = 19.2 mL, desired final volume 25 mL.
   3. (Final Concentration x Final Volume) ÷ Initial Concentration = Initial Volume
      (25 mL x 75,000 cells/mL) ÷ 3,500,000 = 0.536 mL
   4. Volume of FBS in cell suspension. 0.536 mL = 0.536 mL x 0.05 = 0.027 mL
   5. Volume of FBS needed in 25 mL = 25 mL x 0.02 = 0.5 mL

6. Volume of FBS to add to Differentiation Medium = 0.5 mL – 0.026 mL = 0.474 mL
7. Volume of Differentiation Medium = 25 mL – 0.536 mL – 0.474 mL = 24 mL
8. Combine 24 mL Differentiation Medium with 0.474 mL FBS and add 0.536 mL of cell suspension.

Quick Steps For Expansion and Differentiation of AGS-NSC™		
Day 0 (before thawing cells)	1.	Coat culture vessel(s) with Poly-L-Ornithine
	2.	Prepare aliquots of frozen components
	3.	Obtain other components needed for your experiments
Day 1	4.	Prepare AGS-NSC Basal Medium with 5% FBS
	5.	Prepare AGS-NSC Expansion Medium
	6.	Thaw an aliquot of rh FGF-basic
	7.	Thaw and inoculate AGS-NSC into 20 mL of agsNSC Expansion Medium and spike with 40 µL of rh FGF-basic
	8.	Incubate for ~48 hours
Day 3	9.	Replace 100% of the AGS-NSC Expansion Medium in the culture vessel (20 mL) and spike with 40 µL of rh FGF-basic
Day 4	10.	Add 40 µL of rh FGF-basic to the culture vessel (Cells may be ready on day 4 for passaging, especially if this is the second or third passage out of cryopreservation)
Day 5	11.	Cells should be ready to trypsinize (otherwise, continue media changes every other day with daily additions of rh FGF-basic until the cells reach 70% to 80% confluence)
	12.	Prepare AGS-NSC Differentiation Medium
	13.	Trypsinize and count cells as per instructions on page 3
	14.	Inoculate another Poly-L-Ornithine coated T-75 flask with AGS-NSC for further expansion and/or
	15.	Inoculate cells for your experiment into Poly-L-lysine coated 96-well plates
	16.	Two hours after inoculation, perform a 50% medium replacement using AGS-NSC Differentiation Medium
Day 7	17.	Perform a 50% medium replacement using AGS-NSC Differentiation Medium
Day 9	18.	By day 9 (4 days after passaging into Differentiation Medium) cells should be differentiated into immature neurons
	19.	Prepare/obtain Neuronal Maintenance Medium
	20.	Perform a 50% medium replacement using Neuronal Maintenance Medium
	21.	Use cells for experiments within 21 days of inoculation into 96-well plates
Days 12, 15, 18, 21, 24	22.	Perform a 50% media replacement with Neuronal Maintenance Medium
Day 26	23.	Plates should be used or properly disposed by this day

Product	Part No.	Volume	Usage/Comments	Concentration in Supplemented Medium	Storage
AGS-NSC™ Expansion Kit	**SCMA002**	**Kit**	**AGS-NSC Basal Medium (Part# SCMA-BM)** **AGS-NSC Supplement Kit (Part# SCMA002-S)**		
AGS-NSC Basal Medium	SCMA-BM	500 mL			2-8°C
AGS-NSC Expansion Supplement Kit	**SCMA002-S**	**Kit**			**-20°C**
rh FGFb		1.0 mL	Expansion Medium Growth Factor	40 ng/ml	-20°C
Poly-L-Ornithine		25mL	10 µg/mL to coat plates/flasks for expansion	10 ug/ml	-20°C
B27 Supplement		10 mL	Expansion Medium Growth Supplement		-20°C
FBS		10 mL	For neutralization of trypsin		-20°C
AGS-NSC Differentiation Kit	**SCMA003**	**Kit**	**Kit Includes AGS-NSC Basal Medium (Part# SCMA-BM) and ITS-p**		
AGS-NSC Basal Medium	SCMA-BM	500 mL			2-8°C
ITS-*p*		5 mL	Differentiation Medium Growth Factor	10 µg/mL Insulin 5.5 µg/mL Transferrin 0.2 µM Selenium 1.0 mM Pyruvate	-20°C

RELATED PRODUCTS:

Product	*Catalog Code*
Arctic Ground Squirrel NSC Expansion Media Kit	SCMA002
Arctic Ground Squirrel NSC Differentiation Media Kit	SCMA003

This product is for Research Use Only.
This product is not approved for human or veterinary use, or for use in in vitro diagnostic or clinical procedures.

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