Gibco™ human astrocytes and Gibco™ astrocyte medium

Catalog Numbers N7805-200, N7805-100, and A1261301

Doc. Part No. A12625 Pub. No. MAN0002737 Rev. 4.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Description

Gibco™ Human Astrocytes are human brain progenitor-derived astrocytes that are supplied cryopreserved at a concentration of ≥1 × 10^6 cells/mL in Gibco™ Astrocyte Medium without EGF and with 10% DMSO. After thawing, the cells are tested for the astrocyte-specific marker glial fibrillary acid protein (GFAP). See the Certificate of Analysis (COA) for lot-specific results.

Gibco™ Astrocyte Medium (sold separately or as part of a kit with cells) has been specifically formulated for the growth and maintenance of human and rat astrocytes while retaining their phenotype. The medium has three components: basal medium (DMEM), N-2 Supplement, and One Shot™ Fetal Bovine Serum (FBS). Epidermal growth factor (EGF) may also be added to enhance astrocyte proliferation.

Kit name/Components	Catalog no./Part no.	Amount	Storage	Shelf life[1]
Gibco™ Human Astrocyte Kit includes:	**N7805-200**	1 Kit	Liquid nitrogen, vapor-phase	—
Gibco™ Human Astrocytes (≥1 × 10^6 cells/mL)	K1884	1 mL	See below	
Gibco™ Astrocyte Medium	A1261301	1 Kit		
Gibco™ Human Astrocytes (≥1 × 10^6 cells/mL)	**N7805-100** (K1884)	1 mL	Liquid nitrogen, vapor-phase	—
Gibco™ Astrocyte Medium	**A1261301**	5 mL	–5°C to –20°C	18 months
N-2 Supplement, 100X	17502-048	500 mL	2°C to 8°C	12 months
Dulbecco's Modified Eagle Medium (DMEM) (1X)	10569-010 *or* 31966-021 (Europe only)	50 mL	–5°C to –20°C	5 years
One Shot™ Fetal Bovine Serum (FBS), Certified	16000-077			

[1] The shelf life of Complete Astrocyte Medium is 2 weeks at 2°C to 8°C, protected from light.

Important guidelines for thawing and storing cells

- Upon receipt, immediately thaw cells or place into vapor-phase liquid nitrogen storage until ready to use. **Do not store the cells at –80°C**.
- Avoid short-term extreme temperature changes. When storing cells in liquid nitrogen after shipping on dry ice, allow the cells to remain in liquid nitrogen for 3-4 days before thawing.

Precautions

- **Gibco™ Human Astrocytes have limited proliferation ability;** we do not recommend cryopreserving after initial thaw.
- Thaw N-2 Supplement in a 37°C water bath until just thawed; avoid overheating. Use thawed material immediately or store in aliquots (e.g., 1 mL) at –5°C to –20°C. **Avoid additional freeze-thaw cycles**.

Prepare complete astrocyte medium

Gibco™ Astrocyte Medium requires supplementation of DMEM with N-2 Supplement and FBS. Complete Astrocyte Medium is stable for 2 weeks when stored at 2℃ to 8℃ protected from light.

Note: Adding EGF (available separately) at a final concentration of 20 ng/mL can increase astrocyte proliferation, but may result in morphological or phenotypic changes in human astrocytes.

Table 1 Complete medium recipe

Component	100 mL complete medium	500 mL complete medium
DMEM	89 mL	445 mL
N-2	1 mL	5 mL
FBS	10 mL	50 mL
Optional: EGF	2 µg	10 µg

Physical conditions

- Standard physical growth conditions for Gibco™ Human Astrocytes are 36℃–38℃ in a humidified atmosphere of 4–6% CO_2.
- Human astrocytes must be grown on Geltrex™ matrix-coated tissue culture vessels (rat astrocytes may be grown in Complete Astrocyte Medium on standard tissue-culture plates).
- Ensure that Geltrex™ matrix-coated plates are at room temperature for one hour prior to aspirating the media from the stored plates and immediately plate cells in pre-equilibrated Astrocyte Medium. Ensure that proper gas exchange is maintained in culture vessels.
- Avoid overexposure of cultures to light.

Prepare Geltrex™ matrix-coated plates for human astrocytes

Before thawing or passaging Gibco™ Human Astrocytes, prepare culture vessels coated with Geltrex™ matrix as described below.

Note: Rat astrocytes do not require the use of Geltrex™ matrix-coated plates.

1. Thaw a bottle of Geltrex™ Basement Membrane Matrix™ at 2℃ to 8℃ overnight.
2. On ice, prepare a stock solution of Geltrex™ matrix diluted 1:1 in DMEM. Store in aliquots at –20℃ until needed.
3. Dilute the stock solution 1:100 in DMEM and coat the bottom of each culture vessel (200 µL of Geltrex™ matrix per cm^2 of culture vessel).
4. Incubate the culture vessel at 36℃ to 38℃ for 1 hour.

Dishes coated with Geltrex™ matrix can be used immediately or stored at 2℃–8℃ for up to a week, sealed with Parafilm™ laboratory film. Do not allow dishes to dry out.

Note: Warm stored Geltrex™ matrix plates to room temperature for one hour prior to adding astrocytes.

Note: When you are ready to add cells, aspirate the Geltrex™ matrix solution and rinse once with DPBS with calcium and magnesium before adding the cell solution.

Thaw cryopreserved Gibco™ human astrocytes

Before thawing or harvesting Gibco™ Human Astrocytes, prepare culture vessels coated with Geltrex™ matrix as described previously.

IMPORTANT! Astrocytes readily stick to plastics. Prewet all plastics with Complete Astrocyte Medium.

Note: We recommend seeding cells at **4×10^4 cells/cm^2** (360,000 in 2–3 mL in one well of a six well plate).

1. Remove a vial of cells from liquid nitrogen storage and immediately thaw by swirling in a 37℃ water bath. Remove the vial when the last bit of ice has melted, typically <2 minutes. **Do not** (1) submerge the vial completely, (2) thaw for longer than 2 minutes, or (3) create bubbles in the cell suspension, as this will decrease cell viability.
2. When thawed, disinfect the outside of the tube with 70% isopropanol and transfer the tube to a laminar flow hood.
3. Precondition (prewet) a 15-mL centrifuge tube with warm Complete Astrocyte Medium. Discard the medium.
4. Using a prewet sterile pipette tip, slowly (dropwise) transfer the thawed cells (~1 mL) to the preconditioned centrifuge tube.
5. Add 1 mL media to the cryovial. Add this to the centrifuge tube dropwise.
6. Add 3 mL additional of warm Complete Astrocyte Medium dropwise for a total of 5 mL.
7. To remove cryoprotectant (DMSO) from the cells, centrifuge the tube at 290 × g for 5 minutes. Remove and discard the supernatant above the cell pellet.
8. Pre-wet a sterile pipette and suspend the cells in 2–3 mL of warm Complete Astrocyte Medium.
9. Determine the viable cell count using your method of choice (e.g., Countess™ Automated Cell Counter) to seed at the correct density.

   Note: If recovery seems poor, count the cells before and after centrifugation with the next vial to determine if cells are lost due to centrifugation.
10. Adjust the cell density with warm Complete Astrocyte Medium for correct plating density.
11. For human astrocytes, remove a Geltrex™ matrix-coated plate from 2℃ to 8℃ storage and warm to room temperature for one hour. Remove the media by tipping slightly to aspirate the Geltrex™ matrix solution. Rinse the plate once with DPBS with calcium and magnesium.

    Note: Do not allow the plate surface to dry out before plating the cells. (Rat astrocytes do not require Geltrex™ matrix-coated plates.)

12. Immediately plate the cells at 4 × 10^4 cells/cm^2 (360,000 in 2–3 mL in one well of a six well plate).

13. Incubate the cells at 36℃–38℃ in a humidified atmosphere (90%) of 4–6% CO_2 in air. Allow the cells to adhere for at least 24 hours.

    Note: Change the medium every 2 days.

Guidelines for handling and harvesting cells

- **Mature human astrocytes do not significantly proliferate in culture.** The following method can be used to harvest and replate the cells.
- Rat astrocytes *will* proliferate in culture; you can use the following protocol for culturing these cells.
- For optimal performance, media should be changed **every 2 days** with fresh Complete Astrocyte Medium.

Harvesting and replating astrocytes

For replating human astrocytes, prepare culture vessels coated with Geltrex™ matrix as described in the previous section (step 11 on page 3). Equlibrate stored plates to room temperature for one hour prior to use.

1. Warm Complete Astrocyte Medium and StemPro™ Cell Dissociation Reagent in a 37℃ water bath before use.

2. conditioned medium from the cells to a new tube; **this will be used to stop the enzyme reaction in Step 6** on page 3.

3. Wash cells once with 1X DPBS without calcium, magnesium, or phenol red.

4. Aspirate DPBS and add StemPro™ reagent to the cells following the StemPro™ reagent instructions.

5. Incubate for 5–10 minutes at 36℃–38℃. Rock the cells every ~5 minutes and check under a microscope for detachment and dissociation toward single cells.

6. When the cells have detached, add an equal volume (1:1) of conditioned medium (from Step 2 on page 3) to slow the reagent activity.

7. the cells to a 15-mL or 50-mL tube.

8. Rinse culture vessels with 1 mL of Complete Astrocyte Medium and add it to the tube.

9. Centrifuge the tube for 5 minutes at 290 × *g*.

10. Aspirate and discard the supernatant.

11. With a prewet pipette, suspend the pellet in 2–3 mL warm Complete Astrocyte Medium.

12. Count the live cells using a method of choice.

13. To replate human astrocytes, remove a Geltrex™ matrix-coated plate from 2℃ to 8℃ storage and warm to room for one hour. Tip slightly to aspirate the Geltrex™ matrix solution. Rinse the plate once with DPBS with calcium and magnesium.

    Note: Do not allow the plate to dry out.

    Note: Rat astrocytes do not require Geltrex™ matrix-coated plates.

14. Immediately seed the astrocytes at the desired concentration. We recommend 4 × 10^4 cells/cm^2 (360,000 cells in 2–3 mL in one well of a six well plate).

15. Incubate the cells in an incubator at 36℃ to 38℃ in a humidified atmosphere (90%) of 4 to 6% CO_2 in air.

16. Change the medium every 2 days with fresh Complete Astrocyte Medium.

Characterize astrocytes

Astrocytes may be characterized by the following antibodies:

- **Primary Antibody:** Rabbit Anti-GFAP, dilution 1:200
- **Secondary Antibody:** Anti-Rabbit, dilution 1:1000

Images of cells in Gibco™ astrocyte medium

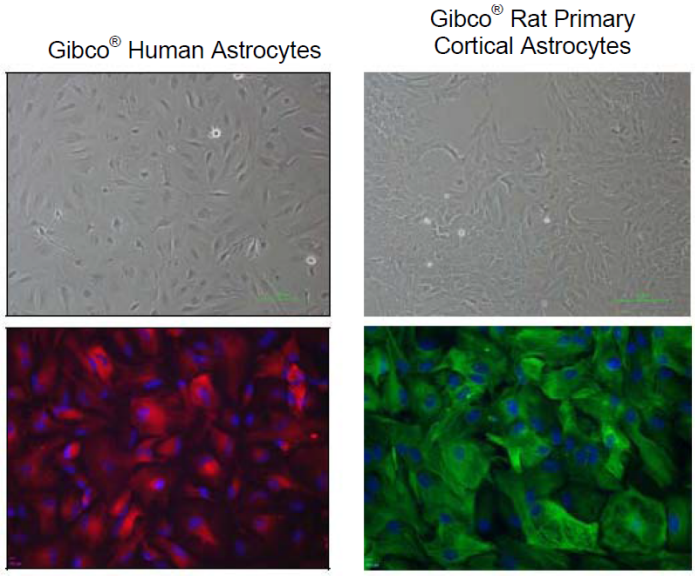

Figure 1 Top: phase images. Bottom: GFAP expression; antibodies used for staining are Alexa Fluor™ 594 goat Anti-Rabbit IgG (human astrocytes) and Alexa Fluor™ 488 goat Anti-Rabbit IgG (rat astrocytes).

Related products

Product	Cat. no.
Gibco™ Rat Primary Cortical Astrocytes	N7745
Geltrex™ Reduced Growth Factor Basement Membrane Matrix™	A14132
StemPro™ Cell Dissociation Reagent	A11105
Dulbecco's Phosphate Buffered Saline (DPBS) with calcium, magnesium (1X), liquid	14040
Dulbecco's Phosphate-Buffered Saline (DPBS), without calcium, magnesium or phenol red (1X), liquid	14190
EGF Recombinant Human	PHG0314

Product	Cat. no.
Fetal Bovine Serum, Qualified	10099
Rabbit Anti-GFAP (Glial Fibrillary Acid Protein)	18-0063
Alexa Fluor™ 488 Goat Anti-Rabbit IgG (H+L)	A11034
Alexa Fluor™ 594 Goat Anti-Rabbit IgG (H+L)	A11037
Trypan Blue Stain 0.4% (for use with the Countess™ Automated Cell Counter)	T10282
Trypan Blue Stain	15250
LIVE/DEAD™ Cell Vitality Assay Kit	L34951
Countess™ Automated Cell Counter	C10227

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Life Technologies Corporation | 3175 Staley Road | Grand Island, NY 14072
For descriptions of symbols on product labels or product documents, go to **thermofisher.com/symbols-definition**.