

Cell Line Designation: HBEC-5i**AddexBio Catalog No. T0005011****Cell Line Description:****Disease:** Normal

Origin: HBEC-5i cells were derived from small fragments of human cerebral cortex obtained from patients who had died of various causes. These brains were devoid of any pathologic abnormalities. Isolation and purification procedures were performed and the cells were cultured. They were then transfected with plasmid containing SV40 large T antigen.

Species: Homo sapiens, human**Tissue:** Brain, cerebral cortex**Properties:** Adherent; cerebral microvascular endothelium**Cytogenic data:** Not available**Patient:** Not available

Complete Medium: DMEM/F12 + endothelial growth supplement (C0010-01; ECGS; 40 µg/mL) or AddexBio DMEM/F12 (HBEC-5i-adapted) (C0013-17) + 10% FBS. Remarks: These cells are cultured on vessels coated with 0.1% Gelatin. Use 1.0 mL of gelatin per 10 cm² surface area. Incubate at 37°C for 45 min. Aspirate gelatin just prior to adding cells to vessel(s).

Subculture Procedure: 1:4 to 1:10 using 0.25% trypsin or trypsin/EDTA, 5% CO₂; 37°C

Medium Renewal: Two to three times weekly.

Freezing Medium: Complete culture medium supplemented with 7.5% (v/v) DMSO

Additional Information: Additional product and technical information can be obtained from the catalog references and the Addexbio Technical Information site at www.addexbio.com, or by email at customersupport@addexbio.com.

Biosafety Level: 2

Appropriate safety procedures should always be used with this material. laboratory safety is discussed in the following publication: Biosafety in Microbiological and Biomedical Laboratories, 5th ed. HHS Publication No. (CDC) 93-8395. U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Washington DC: U.S. Government Printing Office; 2007. The entire text is also available online at www.cdc.gov/od/ohs/biosafety/bmbl4/bmbl4toc.htm

Use Restrictions: These cells are distributed for research purposes only. Addexbio does not recommend third party distribution of this cell line, as this practice has resulted in the unintentional spreading of contaminated cell lines.

Handling Procedure for Frozen Cells:

To insure the highest level of viability, thaw the vial and initiate the culture as soon as possible upon receipt. If upon arrival, continued storage of the frozen culture is necessary, it should be stored in liquid nitrogen vapor phase and not at -70°C. Storage at -70°C will result in loss of viability.

Safety Precaution:

Addexbio highly recommends that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris.

1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the O-ring and cap out of water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions.
3. Transfer and resuspend the vial contents to a T25 flask with recommended complete medium. It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0-7.6).

4. One may also transfer the vial contents into a new culture flask if removal of DMSO is not important. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the complete growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0-7.6).
5. Incubate the culture at 37°C in a suitable incubator for 24-48 hours for cell attachment. A 5% CO₂ in air atmosphere is recommended.

Handling Procedure for Cells in Flask Culture:

The flask was seeded with cells grown and completely filled with complete medium at AddexBio facility that acts as a cushion and to prevent loss of cells during shipping.

1. Upon receipt, carefully examine if the majority of the cells are attached to the bottom of the flask using an inverted microscope (preferably equipped with phase-contrast optics), as the cultures are sometimes handled roughly and many of the cells often detach and become suspended in the culture medium (but are still viable) during shipping. In addition, visually examine the culture for macroscopic evidence of any microbial contamination.
2. **For the cells are still attached**, aseptically remove all but 10 ml of the shipping medium. The shipping medium can be saved for reuse. Incubate the cells at 37°C in a 5% CO₂ in air atmosphere until they are ready to be subcultured.
3. **For the portion of cells that are not attached**, aseptically remove the entire contents of the flask but 10 ml of the shipping medium and centrifuge at 125 x g for 5 minutes. Remove shipping medium and save. Resuspend the pelleted cells in 10 ml of this medium and add to the same 25 cm² flask (T25). Incubate at 37°C in a 5% CO₂ in air atmosphere until they are ready to be subcultured.
4. Cells will need some time to recover from the shipping journey. Keep watching the cells and replace medium (10 ml) every two days without disturbing the monolayer for the first week or until they are 80-85% confluent.

References for HBEC-5i cells:

1. Wassmer SC, de Souza JB, Frère C, Candal FJ, Juhan-Vague I, Grau GE. TGF-beta1 released from activated platelets can induce TNF-stimulated human brain endothelium apoptosis: a new mechanism for microvascular lesion during cerebral malaria. J Immunol. 2006;176(2):1180-1184.



Product Information Sheet for Addexbio

Lot Specific Information Sheet for AddexBio Cat #: T0005011

Lot Number: 0023579

Designation: HBEC-5i cells

Total Cells/mL: $>1.1 \times 10^6$

Expected Viability: 80.1%

Ampule Passage #: 16

Dilute Ampule Content: 1:15 (T-75)

Volume/Ampule: 1.0 mL

A T-75 setup at a dilution of 1:15, using culture medium as described in the product information sheet, reaches approximately 40% confluence within 24 hours.