hESC BG01V

SCRC-2002™

Description

hESC BG01V is a fibroblast cell that was isolated from the inner cell mass of an embryo. hESC BG01V cells are pluripotent and can differentiate into representatives of the three primary germ layers.

Organism: *Homo sapiens*, human
Cell Type: embryonic stem cell
Tissue: Embryo; Inner cell mass
Morphology: spherical colony

Storage Conditions

Product format: Frozen
Storage conditions: Vapor phase of liquid nitrogen

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeutic use, any human or animal consumption, or any diagnostic use.

BSL 1

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current edition of *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, U.S. Department of Health and Human Services. It is your responsibility to understand the hazards associated with the material per your organization's policies and procedures as well as any other applicable regulations as enforced by your local

or national agencies.

Handle as a potentially biohazardous material using universal precautions.

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials. For cultures that require storage in liquid nitrogen, it is important to note that some vials may 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 vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, ATCC recommends that these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitrogen.

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.org.

Growth Conditions

Temperature: 37°C
Atmosphere: 95% Air, 5% CO_2

Handling Procedures

Unpacking and storage instructions:

1. Check all containers for leakage or breakage.
2. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapor, until

ready for use.

Complete medium: 1:1 Mixture of Dulbecco's Modified Eagles Medium and Ham's F-12 medium containing 1.2 g/L sodium bicarbonate, 2.5 mM L-glutamine, 15 mM HEPES and 0.5 mM sodium pyruvate (ATCC 30-2006) supplemented with 2.0 mM L-Alanyl-L-Glutamine (ATCC 30-2115), 0.1 mM Non-essential amino acids (ATCC 30-2116), 0.1 mM 2-mercaptoethanol (Sigma Catalog No. M-7522) and 4 ng/ml bFGF (R& D Systems Catalog No. 233-FB), 80%; Knockout serum replacement (Invitrogen Catalog No. 10828), 5%; fetal bovine serum (ATCC SCRR-30-2020), 15%

Feeder cells: Feeder cells may be grown in medium containing fewer growth factors than those required by the ES cells. Feeder cells are available from ATCC. Consult the product detail page and the product sheet provided for the feeder cells you wish to use for medium requirements. Feeder cells should be initiated 24 to 48 hours prior to inoculating with embryonic stem (ES) cells.

Handling Procedure:

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.

To insure the highest level of viability, be sure to warm media to 37ºC before using it on the cells. The cells were frozen in clumps since wild type Human ES cells experience low viability when dissociated to single cells.

1. Plate radiation treated mouse embryonic fibroblasts (MEF, SCRC-1040.1) as a feeder layer onto appropriate size flask at least one day before thawing the vial. Use Table 1 to determine the correct density of feeders to plate (see product sheet for SCRC-10401.1 for protocol). One hour before thawing the vial of ES cells, perform a 100% medium change using complete growth medium (see below for recipe).

Table 1. Plating Densities for MEFs

Flask/Plate	Growth Area (cm^2)	CF-1 MEFs
T_{225}	225	18.0×10^6

T_{75}	75	7.0 x 10^6
T_{25}	25	2.5 x 10^6
6 well	9.5	0.5 x 10^6

2. 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 the water. Thawing should be rapid (approximately 90 seconds).
3. Remove the vial from the water bath before the cells are completely 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.*
4. Transfer the vial's contents plus 4 mL of complete growth medium to a 15 mL centrifuge tube. Use an additional 1 mL of media to rinse the vial and transfer the liquid to the 15 mL tube. Add 4 ml of complete growth medium to bring the total volume to 10 mL.
5. Spin the cells at 270 x g for 5 minutes.
6. Aspirate the supernatant and resuspend the pellet in 3 ml of complete medium.
7. Add the 3 mL of cell suspension to one T25 flask or 3 wells of a 6 well plate (1 mL/well) containing feeders and growth medium.
8. Incubate the culture at 37°C in a humidified 5% CO_2/95% air incubator.

Do not change the medium for the first 48 hours. However, add an additional 4 ng/mL of bFGF 24 hours after the thaw. After the first 48 hours, change the medium daily.

Examine the colonies daily using an inverted microscope. It can take up to one week for colonies to appear. The first passage should occur 3-4 days after colonies are visible.

Subculturing procedure:

To insure the highest level of viability, be sure to warm media to 37°C before using it on the cells. The passaging ratio depends on the density/confluency of the colonies. It ranges between 1:3 and 1:6. *Note: If the colonies are close to or touching each other the culture is overgrown. Overgrowth will result in differentiation.*

1. At least 24 hours prior to each passage, plate treated MEFs onto the culture

vessels to be used. Base the number of dishs/flasks to be used on the passaging ratio. Refer to Table 1 to determine the correct plating density for the feeders.

2. Prepare 0.5 mg/mL or ~200 units/mL Collagenase IV solution (Invitrogen 17104-019) in DMEM/F12 and sterile filter using 0.22 µm low-protein binding filter. Check the units/mg for each lot of powder.
3. Remove medium from cells. Add appropriate volume of Collagenase IV solution. Refer to Table 2 to determine the correct amount.
4. Incubate at 37°C for up to 2 hours.
5. Check the cells after the first 30 minutes and then every 15 minutes. When the majority of the hESC colonies have completely detached or the edges of the colonies have rounded up, add appropriate amount of DMEM/F12 (Table 2) and wash gently using a pipette. Under optimal conditions, all the colonies can be washed off with feeder cells left behind. If some colonies are still attached, gently scrape the surface area with the tip of a 5 mL pipette if necessary.
6. Collect cell suspensions into a 50 mL conical tube.
7. Centrifuge for 5 minutes at 200 x g at 25°C.
8. Remove the supernatant and resuspend in complete growth medium. Pipette up and down to break the colonies to smaller clumps and evenly distribute cells to feeder-covered dishes/flasks.
9. Add complete growth medium to each tissue culture vessel to achieve the appropriate final volume. Refer to Table #2 to find the appropriate volume based on surface area.

Table 2. Reagent Quantities

Flask/Plate	Growth Area (cm^2)	Collagenase (ml)	DMEM/F12 (ml)	Growth Medium (ml)
T_{225}	225	10	10	30
T_{75}	75	3.0	5	12
T_{25}	25	2	5	6
6 well	9.5	0.5	1	3

Medium Renewal

Every day after the first 48 hours

Complete Growth Medium for Feeder Cells

The feeder cells are grown in DMEM (ATCC #30-2002) supplemented with 15% FBS (ATCC # 30-2020).

Cryopreservation:

1. Follow the Subculturing Procedure above and use Collagenase IV to dissociate the cells.
2. Centrifuge the cell suspension for 5 minutes at 200xg at 25°C.
3. Resuspend the pellet in a 1:1 solution of 50% complete growth medium and 50% FBS. The total volume should be 0.5 ml times the number of vials to be frozen. Determine the number of vials using Table 3.
4. Pipette up and down to break the colonies into small clumps. P1000 tips are used to efficiently break up the colonies.
5. Slowly add an equal volume of complete growth medium with 20% DMSO. Mix gently.
6. Evenly distribute 1 mL of the cell suspension into each cryovial.
7. Store the vials in Styrofoam boxes at -80°C. Transfer the vials to liquid nitrogen 24 hours later.

Table 3. # of Vials at 80-90% confluency

Flask/Plate	Growth Area (cm^2)	# of vials
T_{225}	225	16
T_{75}	75	5
T_{25}	25	2
6 well	9.5	1

Cryoprotectant Medium: Complete growth medium supplemented with 20% FBS and 10% DMSO. Follow the two step process in the Cryopreservation protocol. Cell-culture tested DMSO is available as ATCC Catalog No. 4-X.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: hESC BG01V (ATCC SCRC-2002)

References

References and other information relating to this material are available at www.atcc.org.

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