ATCC

Product Sheet

hESC BG01V (ATCC® SCRC-2002™)

Please read this FIRST

Storage Temp.
liquid nitrogen vapor phase

Biosafety Level
1

Intended Use

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

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

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: hESC BG01V (ATCC® SCRC-2002™)

American Type Culture Collection
PO Box 1549
Manassas, VA 20108 USA
www.atcc.org

800.638.6597 or 703.365.2700
Fax: 703.365.2750
Email: Tech@atcc.org

Or contact your local distributor

Description

Organism: *Homo sapiens*, human
Tissue: inner cell mass
Cell Type: embryonic stem cell
Age: embryo, blastocyst
Morphology: spherical colony
Cytogenetic Analysis: 49, XXY, +12, +17

Batch-Specific Information

Refer to the Certificate of Analysis for batch-specific test results.

SAFETY PRECAUTION

ATCC 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.

Unpacking & 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.

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.
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	7.0×10^6
T_{25}	25	2.5×10^6
6 well	9.5	0.5×10^6

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.

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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 (cm²)	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 Medium

To freeze the cells:

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 25ºC.
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 -80ºC. Transfer the vials to liquid nitrogen 24 hours later.

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

Flask/Plate	Growth Area (cm²)	# 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.

Comments

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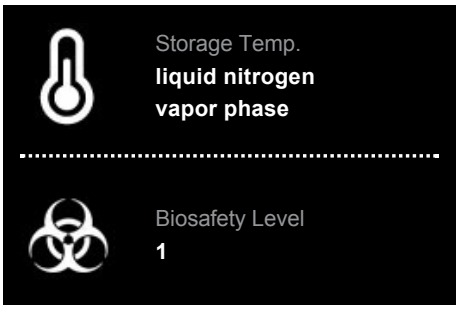

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BG01V is a human embryonic stem cell line with an abnormal karyotype. Despite the abnormal karyotype, when grown on murine embryonic feeders (MEFs) these colonies exhibit uniform morphology, a predictable growth rate, and are easy to maintain in culture. The cells stain positive for pluripotency markers and alkaline phosphatase activity.

References

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

Biosafety Level: 1

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the current publication of the *Biosafety in Microbiological and Biomedical Laboratories* from the U.S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes for Health.

ATCC Warranty

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Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.
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Please see the enclosed Material Transfer Agreement (MTA) for further details regarding the use of this product. The MTA is also available on our Web site at www.atcc.org

Additional information on this culture is available on the ATCC web site at www.atcc.org.

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