ATCC

Product Sheet

DSDh (ATCC® CRL-2131™)

Please read this FIRST

Biosafety Level
2

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

Minimum essential medium (Eagle) with Earle's BSS containing 500 nM methotrexate, 92%; fetal bovine serum, 8%

Citation of Strain

If use of this culture results in a scientific publication, it should be cited in that manuscript in the following manner: DSDh (ATCC® CRL-2131™)

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: *Canis familiaris*, dog
Strain: poodle
Tissue: bone
Disease: osteosarcoma
Age: 11 years
Gender: female
Morphology: fibroblast
Growth Properties: adherent

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

HANDLING PROCEDURE FOR FROZEN CELLS

- Initiate culture as soon as possible upon receipt.

- Thaw by rapid agitation in 37°C water bath. Thawing should be rapid (within 40-60 seconds). As soon as the ice is melted, remove the ampule from the water bath and immerse in 70% ethanol at room temperature. All of the operations from this point on should be carried out under strict aseptic conditions.

- Transfer the cell suspension and dilute it with the recommended culture medium in a culture flask (see specific batch information above for dilution ratio); incubate at 37°C with 5% CO_2 in air atmosphere. Since it is important to avoid excessive alkalinity of the medium during recovery of the cells, it is suggested that the culture medium be placed into the culture flask, tube, etc. and the pH be adjusted, as necessary, prior to the addition of the ampule contents. Note that the bicarbonate content of the culture medium will determine whether an atmosphere containing CO_2 will be required.

- It is not necessary to remove the freezing additive. However, if desired, the culture medium may be changed to remove the protective freezing additive (dimethylsulfoxide) 24 hours after thawing. If it is desired that the freezing additive be removed immediately, or that a more concentrated cell suspension be obtained, centrifuge the above diluted suspension at approximately 125 x g for 10 minutes, discard the fluid and resuspend the cells with growth medium at the dilution ratio given in the specific batch information above.

FLUID RENEWAL
Twice weekly.

SUBCULTURE PROCEDURE
Remove medium, rinse with trypsin (0.25%) - EDTA (0.03%) solution. Add 1-2 fresh trypsin and allow flasks to remain at room temperature (or incubate at 37°C) until cells detach (approximately 3-5 minutes). Add fresh culture medium, aspirate and dispense into new culture flasks. Inoculate new culture flasks at

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approximately 1-2 x 10(5) cells/sq. cm. Transfer cells at or before confluency.

Handling Procedure for Flask Cultures

HANDLING PROCEDURE FOR FLASK CULTURES (MONOLAYER)
The flask was seeded with cells (see specific batch information above for concentration), grown and completely filled with medium to prevent loss of cells in transit. Remove all of the medium (which can be saved and used as fresh medium) except for a sufficient volume (5-10 ml) to cover the floor of the flask. Incubate at 37°C in a 5% CO_2 in air atmosphere. Sometimes in transit the cultures are handled roughly and most of the cells become detached and float in the culture medium. If this has occurred remove the entire contents of the flask and centrifuge at 300 x g for 15 minutes. Draw off the excess supernatant medium, resuspend the cells in 10 ml of the culture medium and plant the entire cell suspension in a single flask of suitable size (about 25 sq. cm.).

Subculturing Procedure

Medium Renewal: Twice per week
Remove spent medium, add fresh 0.25% trypsin, 0.03% EDTA solution, rinse and remove trypsin.
Add fresh trypsin solution (1 to 2 ml) and let the culture sit at room temperature (or at 37C) until the cells detach.
Add fresh medium, aspirate and dispense into new flasks.
Subculture at or prior to becoming confluent.
Inoculate new flasks with 1 to 2 X 10 exp5 cells per sq cm.

Comments

DSDh is a retrovirus packaging cell line derive from the D-17 canine osteogenic sarcoma cell line (ATCC CCL-183) by Howard Temin.
D-17 cells were transfected with plasmids pBR1 (gag - pol genes from spleen necrosis virus), pPR102 (env gene from spleen necrosis virus) and pFR400 (dihydrofolate reductase gene).
DSDh cells were isolated after selection with methotrexate and screening for helper activity.
The line is used as a helper cell for propagating replication incompetent vectors derived from spleen necrosis virus (SNV).
After serial passaging for 2 months, the cells appears to decrease in helper activity (as evidence by drops in viral titers produced), thus it is best to prepare generous frozen stocks of the cells upon receipt.

References

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

Biosafety Level: 2

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

ATCC® products are warranted for 30 days from the date of shipment, and this warranty is valid only if the product is stored and handled according to the information included on this product information sheet. If the ATCC® product is a living cell or microorganism, ATCC lists the media formulation that has been found to be effective for this product. While other, unspecified media may also produce satisfactory results, a change in media or the absence of an additive from the ATCC recommended media may affect recovery, growth and/or function of this product. If an alternative medium formulation is used, the ATCC warranty for viability is no longer valid.

Disclaimers

This product is intended for laboratory research purposes only. It is not intended for use in humans.
While ATCC uses reasonable efforts to include accurate and up-to-date information on this product sheet, ATCC makes no warranties or representations as to its accuracy. Citations from scientific literature and

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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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