



Designation: **AH-130**

CLS order number: Cryovial: 500412
 Vital: 550412

Origin and General Characteristics	
Depositor:	CLS
Organism:	<i>Rattus norvegicus</i> (rat)
Strain:	Sprague-Dawley
Tissue:	Hepatoma
Morphology:	Round suspension cells, triangular adherent cells
Growth Properties:	Mixed culture - suspension/monolayer. Semi-adherent.
Description:	Yoshida et al. have established the ascites hepatoma by converting the aminoazo dye-induced hepatoma of the rat into the ascetic form (Yoshida 1956). AH-130 is a strain of ascites hepatoma composed of free tumor cells, only small islands of tumor islets are present. The cell line described here was established as in vitro cell culture from this Yoshida AH-130 strain of ascites hepatoma.
References:	Yoshida T. Ann. N.Y. Acad.Sci. 63: 862, 1956. Hoshino M. Submicroscopic characteristics of four strains of Yoshida ascites hepatoma of rats: a comparative study. Cancer Res. 23:209-16, 1963. Feo F et al. Further observations on the effect of trypsin on the volume and functions of mitochondria isolated from normal liver and AH-130 Yoshida ascites hepatoma. Cancer Res 33: 1804-12, 1973.
Culture Conditions and Handling	
Culture Medium:	DMEM:Ham's F12 medium (1:1 mixture) supplemented with 2 mM L-glutamine and 5% fetal bovine serum (MG-40, CLS order number 820400).
Subculturing:	Collect the culture medium in a tube and rinse the adherent cells using PBS without calcium and magnesium (3-5 ml PBS for T25, 5-10ml for T75 cell culture flasks). Add Accutase (1-2ml per T25, 2.5ml per T75 cell culture flask), the cell sheet must be covered completely. Incubate at ambient temperature for 8-10 minutes. Carefully resuspend the cells with medium (10 ml), centrifuge together with the suspension cells for 3 min at 300xg, resuspend cells in fresh medium and dispense into new flasks which contain fresh medium. Using collagen-coated flasks increases the number of adherent cells.
Split Ratio:	A ratio of 1:2 to 1:4 is recommended
Seeding density:	2x10 ⁴ /cm ²
Fluid Renewal:	every 3 to 5 days
Freeze Medium:	CM-ACF (CLS order number: 800650, 50ml)
Freezing recovery:	Fast
Sterility:	Plasmotest: negative; Mycoplasma specific PCR: negative
Biosafety Level:	1
Safety precautions:	If the cryovial is planned to be stored in liquid nitrogen and to be thawed in the future, special safety precautions should be followed: Protective gloves and clothing should be used and a facemask or safety goggles must be worn when transferring frozen samples into or removing from the liquid nitrogen tank. The removal of a cryovial from liquid nitrogen may result in the explosion of the frozen vial creating flying fragments. Caputo, J.L. Biosafety procedures in cell culture. J. Tissue Cult. Methods 11:223-227, 1988. ATCC Quality Control Methods for Cell Lines, 2nd edition, 1992.

Special Features of the Cell Line	
Tumorigenic:	Yes, in Wistar and other strains.
Viruses:	RAP-Test negative. SMRV: Negative, as confirmed by Real-Time PCR.
Virus Susceptibility:	Very sensitive to human adenoviruses
Authentication :	The rat origin was verified by Real-Time PCR.

Certificate of Analysis:	The Certificate of Analysis for each batch can be requested by e-mail at service@clsgmbh.de .
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Recommendations for handling of adherent cell cultures following delivery	
Cryopreserved cells	<p>The cells come deep-frozen shipped on dry ice. Please make sure that the vial is still frozen.</p> <p>If immediate culturing is not intended, the cryovial(s) must be stored below -150°C after arrival.</p> <p>If immediate culturing is intended, please follow these instructions:</p> <p>Quickly thaw by rapid agitation in a 37°C water bath within 40-60 seconds. The water bath should have clean water containing an antimicrobial agent. As soon as the sample has thawed, remove the cryovial from the water bath. Note: A small ice clump should still remain and the vial should still be cold.</p> <p>From now on, all operations should be carried out under aseptic conditions.</p> <p>Transfer the cryovial to a sterile flow cabinet and wipe with 70% alcohol. Carefully open the vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of culture medium (room temperature). Resuspend the cells carefully. Centrifuge at 300xg for 3 min and discard the supernatant. The centrifugation step may be omitted, but in this case the remains of the freeze medium have to be removed 24 hours later.</p> <p>Resuspend the cells carefully in 10ml fresh cell culture medium and transfer them into one T25 cell culture flask. All further steps are described in the Subculture section.</p>
Proliferating Cultures	<p>The cell culture flask, 2xT25, come filled with cell culture medium.</p> <p>Incubate at 37°C for a minimum of 24 hrs.</p> <p>Count the cells, spin down the cell suspension at 300x g for 3 minutes to collect the cells. Resuspend the cells in an appropriate amount of fresh cell culture medium and transfer to new cell culture flasks.</p> <p>Incubate at 37°C for a minimum of 24 hrs.</p>

Warranty:	CLS warrants for a high cell viability and culture performance only if the product(s) is (are) stored and cultured according to the information described above. Using cell culture media and supplements other than the ones recommended in this product information may result in satisfactory proliferation and viabilities. CLS, however, does not warrant for cell recovery, proliferation and function if differing formulations are employed.
Disclaimer:	The customer shall not be entitled to employ this product for purposes other than research. Commercial utilization shall not be permitted; in particular, the cell line, its components or materials made therefrom shall not be sold or transferred to any third party. In addition, the term 'Commercial use' shall mean any activity by a party for consideration and may include, but is not limited to, use of the product or its components in manufacturing, for providing services, e.g. fee for service testing, in quality control or assurance processes within the manufacturing of products for sale, for therapeutic, diagnostic or prophylactic purposes, or for resale.