Designation: **LMH**

CLS order number: Cryovial: 601411
Vital: 661411

Origin and General Characteristics	
Organism:	*Gallus gallus* (chicken)
Strain:	Leghorn
Tissue:	Liver
Morphology:	Epithelial; Dendritic like.
Cell type:	Hepatocellular carcinoma; induced using diethylnitrosamine.
Growth Properties:	Adherent
Description:	The cell line LMH was established in 1981 by Tomoyuki Kitagawa at the Cancer Institute, Kami-Ikebukuro-ku of Tokyo, Japan. Primary hepatocellular carcinoma epithelial cell lines were transformed into permanent cells by inducing tumorous nodules by long term treatment with diethylnitrosamine in the liver of a male leghorn cchicken. LMH cells are inducible for the expression of the liver specific apolipoprotein II (apoll) gene.
References:	Kawaguchi T et al. Establishment and Characterization of a Chicken Hepatocellular Carcinoma Cell Line, LMH. Cancer Res 47: 4460-4, 1987.
Culture Conditions and Handling	
Culture Medium:	EMEM supplemented with L-glutamine, 10% fetal bovine serum (MG-10, CLS order number 820100). Alternative: Waymouth`s MB 752 medium supplemented with L-glutamine and 10% fetal bovine serum.
Subculturing:	Remove medium 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 ambiente temperature for 8-10 minutes. Carefully resuspend the cells with medium (10 ml), centrifuge for 5 min at 300xg, resuspend cells in fresh medium and dispense into new flasks which contain fresh medium. **LMH cells attach better to tissue culture vessels which have been precoated with Collagen.**
Split Ratio:	A ratio of 1:2 to 1:4 is recommended
Seeding density:	1-3 x 10^5 cells/cm^2
Fluid Renewal:	2 times weekly
Freeze Medium:	CM-1 (CLS order number 800150, 50ml)
Sterility:	Cell based assay (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:	LMH cells form tumors in athymic mice.

Viruses:	SMRV: Negative, as confirmed by Real-Time PCR
Karyotype:	triploid; modal number = 116; six marker chromosomes
Receptors Expressed:	Estrogen (low level expression).
Released factors:	glucose-6-phosphatase; canalicular ATPase activity (weak)
Applications:	The cell line is useful for transfection studies.

Certificate of Analysis:	The Certificate of Analysis for each batch can be requested by e-mail at service@clsgmbh.de.

Recommendations for the handling of adherent cells following delivery	
Cryopreserved cells:	The cells come deep-frozen shipped on dry ice. Please make sure that the vial is still frozen. If immediate culturing is not intended, the cryovial(s) must be stored below -150°C after arrival. If immediate culturing is intended, please follow these instructions: 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. From now on, all operations should be carried out under aseptic conditions. 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. Resuspend the cells carefully in 10ml fresh cell culture medium and transfer them into two T25 cell culture flasks. All further steps are described in the Subculture section.
Proliferating Cultures:	The cell culture flasks, 2xT25, come filled with cell culture medium. Collect the entire medium in 2x 50 ml centrifuge tubes. Carefully add 5 ml of cell culture medium to each of the two T25 cell culture flasks. Control the cell morphology and confluency under the microscope. Incubate at 37°C for a minimum of 24 hrs. Spin down the collected medium at 300x g for 3 minutes to collect the cells which may have detached during transit. If a cell pellet is visible, resuspend the cells in 5 ml of cell culture medium and transfer to 1xT25 cell culture. Incubate at 37°C for a minimum of 24 hrs.

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.