Designation: **Hep-55.1C**

CLS order number: Cryovial: 400201
Vital: 440201

Origin and General Characteristics	
Organism:	Mouse (Mus musculus)
Strain:	C57BL/6J
Age/Stage:	Adult
Gender:	Female
Tissue:	Liver
Morphology:	Epithelial
Cell type:	Hepatoma
Growth Properties:	Monolayer, adherent
Description:	Established from the primary hepatocellular carcinoma of C57BL/6J mice.
References:	Kress S et al. p53 mutations are absent from carcinogen-induced mouse liver tumors but occur in cell lines established from these tumors. Molecular carcinogenesis 6: 148-158, 1992.
Culture Conditions and Handling	
Culture Medium:	DMEM supplemented with 4.5 g/L glucose, 2 mM L-glutamine and 10% fetal bovine serum (MG-30, CLS order number 820300).
Culturing process:	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 ambient temperature for 8-10 minutes. Carefully resuspend the cells with medium (10 ml), centrifuge for 3-5 min at 300xg, resuspend cells in fresh medium and dispense into new flasks which contain fresh medium.
Split Ratio:	A ratio of 1:4 to 1:8 is recommended
Fluid Renewal:	Every 3 to 5 days
Freeze Medium:	CM-1 (CLS order number: 800125, 25ml, 800150, 50ml)
Freezing Recovery:	Start culture from cryovial at a cell density of 3-4x10^4 cells/cm^2. The cells will recover within 24-48 hrs.
Sterility:	Fluorescence (DAPI) test: negative; Mycoplasma specific PCR: negative; Bacteria 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 C57BL/6J mice
Viruses:	SMRV: Negative, as confirmed by Real-Time PCR
Authentication:	The mouse origin was verified by Real-Time PCR.
Protein pattern:	Keratin 8, keratin 18, Vimentin
Mutations:	p53 wt
Certificate of Analysis:	The Certificate of Analysis for each batch can be requested by e-mail at service@clsgmbh.de.

Recommendations for handling of adherent cell cultures following delivery	
Cryopreserved cells:	If immediate culturing is not intended, the cryovial(s) must be stored below -150°C or at least at -80°C after arrival. Do not store at -80°C for more than 1-2 days. 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.
Proliferating Cultures:	The cell culture flasks are completely filled with cell culture medium to prevent loss of cells during transit. Remove the entire medium except for a sufficient volume to cover the floor of the flask. Incubate at 37°C for 24 hrs. Sometimes the cultures are handled roughly during transit, and most of the cells detach and float in the culture medium. If this has occurred remove the entire content of the flask and centrifuge at 300x g for 3 minutes. Take off the supernatant, resuspend the cells in 10 ml of culture medium and transfer the entire cell suspension into 2xT25 cell culture flasks.

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 unless confirmed on the basis of a MTA. 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.