Designation: **KHOS-312H**

CLS order number: Cryovial: 300447
Vital: 330447

Origin and General Characteristics	
Organism:	Homo sapiens (human)
Ethnicity:	Caucasian
Age:	13 years
Gender:	Female
Tissue:	Bone
Cell type:	Sarcoma, osteogenic
Growth Properties:	Monolayer, adherent
Description:	The growth properties of KHOS-312H are similar to HOS (TE-85). The KHOS-312H does not represent a rescuable Kirsten murine sarcoma virus genome.
References:	Cho HY et al. Revertants of human cells transformed by murine sarcoma virus. Science 194: 951-3, 1976.
Culture Conditions and Handling	
Culture Medium:	Minimum essential medium Eagle (Earle's BSS) supplemented with 2 mM L-glutamine, 10% fetal bovine serum (MG-10, CLS order number 820100).
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 ambient temperature for 10 minutes. Carefully resuspend the cells with medium (10 ml), centrifuge for 3 min at 300xg, resuspend cells in fresh medium and dispense into new flasks which contain fresh medium.
Split Ratio:	A ratio of 1 to 3 is recommended
Seeding density:	$1x10^4$ cells/cm^2
Fluid Renewal:	2 to 3 times weekly
Freeze Medium:	CM-ACF (CLS order number 800650, 50ml)
Freezing recovery:	After thawing, plate the cells at 5 x 10^4 cells/cm^2 and allow the cells to recover from the freezing process and to adhere for at least 24 hrs.
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:	No
Viruses:	SMRV: Negative, as confirmed by Real-Time PCR

DNA Profile (STR):	Amelogenin: X,X CSF1PO: 12 D13S317: 12 D16S539: 10,13 D5S818: 13 D7S820: 11,12 THO1: 6 TPOX: 8,11	vWA: 18 D3S1358: 15 D21S11: 31.2,32.2 D18S51: 14,17 Penta E: 7,12 Penta D: 9,10 D8S1179: 11,14 FGA: 24

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	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 containing the adherent KHOS-312H cells are completely filled with cell culture medium to prevent loss of cells during transit. Collect the entire medium in sterile 50 ml tubes except for a sufficient volume to cover the bottom of the flasks. Control if the cells adhere at the bottom of the flasks. If the cell sheet covers at least 50% of the bottom, discard the supernatant. If you notice lower confluence, continue with collecting the cells in the supernatant. Centrifuge the supernatant for 3 min at 300xg (please do not exceed this centrifugation force), remove the medium and resuspend the pellet in 5 ml cell culture medium. Transfer the 5 ml into 1x T25 cell culture flask. Incubate the T25 cell culture flasks at 37°C/5% CO_2 for at least 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.