Designation: **CTLL-2**

CLS order number: Cryovial: 400482
genomic DNA: 400482GD5, 5 µg
Snap-frozen cell pellet: 400482CP
Whole cell lysate: 400482CL, 250 µg

Origin and General Characteristics	
Organism:	*Mus musculus* (mouse)
Strain:	C57BL/6
Tissue:	Blood
Morphology:	Single cell suspension; round, shining cells
Cell type:	Lymphoblast
Growth Properties:	Suspension
Description:	The CTLL-2 cell line is a clone of cytotoxic T cells collected from repetitive allogeneic MTLC (Mixed Tumour-Lymphocyte Cultures) of spleen cells from C57BL/6 mice immunised with F4-5 Friend virus (FLV)-induced leukemia cells. The cells require the presence of IL-2 in the cell culture medium otherwise they will ceise proliferating.
References:	Gillis S , Smith KA . Long term culture of tumour-specific cytotoxic T cells. Nature 268: 154-156, 1977. PMID: 145543; DOI=10.1038/268154a0.
Culture Conditions and Handling	
Culture Medium:	1. i2Cult (Immunservice, Germany) complete medium ready-to-use. This medium is highly recommended as due to its special formulation CTLL-2 cells are optimally fed and grow exceptionally well, as proven by Cell Lines Service. 2. RPMI 1640 medium supplemented with 2 mM L-glutamine, 10% fetal bovine serum and 10-20 IU/ml Human recombinant IL-2 of any provider (CLS order number 840010 for RPMI 1640, 840903-50 for FBS, EU-compliant).
Subculturing:	Immediately after thawing, about 50% viable cells were measured using Trypan Blue dye exclusion. The viability of the cells eventually will drop to even lower values. Using medium i2Cult, cell viability increased to > 80% within 48 hrs, at a cell concentration of about 1 Mio. cells/ml. Subculture the cells at an inoculation density of 40.000 cells/ml. Control the cell viability every day. Keep the cells at 37°C and 5% CO_2.
Split Ratio:	A ratio of 1:50 to 1:100 is recommended.
Fluid Renewal:	Every 2 to 3 days
Freeze Medium:	CM-1 (CLS order number: 800125, 25ml, 800150, 50ml)
Freezing recovery:	After thawing, allow the cells to recover from the freezing process for at least 24 hrs.
Sterility:	Mycoplasma specific PCR: negative
Biosafety Level:	1 According to Gillies et al., F4-5 are non-virus-producing murine leukemia cells as shown by reverse transcriptase assay.
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	
Viruses:	Tested and found negative for ectromelia virus (mousepox); SMRV: Negative, as confirmed by Real-Time PCR.
Karyotype:	Not specified
Authentication :	The mouse origin was verified by Real-Time PCR.
Receptors expressed:	IL-2

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

Recommendations for handling of cells growing in suspension 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 one T25 cell culture flask. All further steps are described in the Subculture section.
Proliferating Cultures	The cell culture flask, 1xT25, comes filled with cell culture medium. Incubate at 37°C for a minimum of 24 hrs. 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. 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.