Designation: **Sf9**

CLS order number: Cryovial: 604328
Vital: 664328

Origin and General Characteristics	
Organism:	Spodoptera frugiperda (fall armyworm)
Stage:	Pupa
Gender:	Female
Tissue:	Ovary
Morphology:	Round, attached, epitheloid
Growth Properties:	Adherent
Description:	The Sf9 cell line is a suitable host for the expression of recombinant proteins using baculovirus expression vectors.
Culture Conditions and Handling	
Culture Medium:	Medium for Sf9 cells is available from different suppliers; for example Spodopan (PAN Biotech) in recommended; addition of 2% FBS enhances proliferation. Use cold medium for medium exchanges or seeding of cells.
Subculturing:	Detachment of cells via a cell scraper is recommended. Collect the medium with detached cells after scraping in a 15ml centrifuge tube. Add about 5ml of medium to the flask and rinse the flask several times to collect any remaining cells and combine them with the rest of the cells in the tube. Centrifuge for 3 min at 300xg, remove the supernatant, resuspend the cells in fresh, cold medium and dispense into new flasks.
Split Ratio:	For the first two subcultivations a ratio of 1:3 to 1:5 is recommended; in further subcultivations cells can be split at a ratio of 1:10 to 1:20.
Maintenance:	Incubate between 26 to 30°C in a non-humidified, ambient air-regulated incubator. Use cell culture flasks with filter caps or loosen caps to allow for oxygen exchange.
Seeding density:	10,000 cells / cm^2.
Fluid Renewal:	2-3 times weekly
Freeze Medium:	Animal-component free CM-ACF (CLS order number 800650, 50ml or 800625, 25 ml)
Sterility:	Cell based 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	
Virus susceptability:	Baculoviruses; Autographa californica (MNPV); St. Louis encephalitis (SLE)
Applications:	Transfection host

References:

Vaughn JL et al. The establishment of two cell lines from the insect Spodoptera frugiperda (Lepidoptera; Noctuidae). In Vitro 13: 213-7, 1977.

Lynn, Dwight E. Methods for maintaining insect cell cultures. 6 pp. J of Insect Science, 2.9., 2002.

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 Sf9 cells	
Thawing of Frozen cells:	Quickly thaw by rapid agitation in a 37°C water bath within 1-3 minutes. 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 (a small ice clump should remain and the cryovial should still be cold). All operations from now on 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 resuspend the cells in 8 ml of cell culture medium in a 15-ml-tube. Remove the cryoprotectant by centrifugation (300xg, 3 minutes). Carefully take off the supernatant, resuspend the cells in 10 ml of fresh medium and pipette into two T25 cell culture flasks. Incubate between 26 to 30°C in a non-humidified, ambient air-regulated incubator. Use cell culture flasks with filter caps or loosen caps to allow for oxygen exchange. If the cells grow well and reach confluence (or post-confluence, optimum 3 to 4 days past confluence), split into T75 cell culture flask(s) containing 15 ml of medium. Viability of Sf9 cells after thawing can be low, i.e. 45% to 65% have been observed, regularly. However, the cells recover within 4 to 5 days to a confluence of 80 to 100%.
Proliferating cultures:	For transport, the cell culture flasks have been completely filled with culture medium to prevent loss of cells during transit. After delivery, collect the entire medium in 2x 50 ml centrifuge tubes. Carefully add 5 ml of fresh cell culture medium to each of the two T25 cell culture flasks. Control the cell morphology and confluency under the microscope. 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 between 26 to 30°C in a non-humidified, ambient air-regulated incubator. Use cell culture flasks with filter caps or loosen caps to allow for oxygen exchange and incubate for at least 24 to 48 hrs. At confluence, perform the steps as described under Culture Conditions and Handling – Subculturing.
Freezing procedure:	The cultures should be about 80-90% confluent; detach the cells according to the protocol described above. Count the cells and collect the cells at 300xg for 3 minutes. Resuspend the cells at a concentration of 3.3 million/ml of freezing medium (e.g. CM-ACF, CLS order number 800650, 50ml or 800625, 25 ml). Aliquot the cells in sterile cryovials (5 million/vial) and put them at -20°C for 40 minutes. Place the cryovials overnight at -80°C into a deep-freezer. For long term storage, transfer the frozen vials into a liquid nitrogen container. Storage of the frozen vials at -80°C for more than 2-3 days is not recommended.

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. This product from CLS has been manufactured under license for third parties. The customer shall take the submitted licensing terms and conditions of third parties into consideration and may only make use of such within the scope of the rights granted therein.