

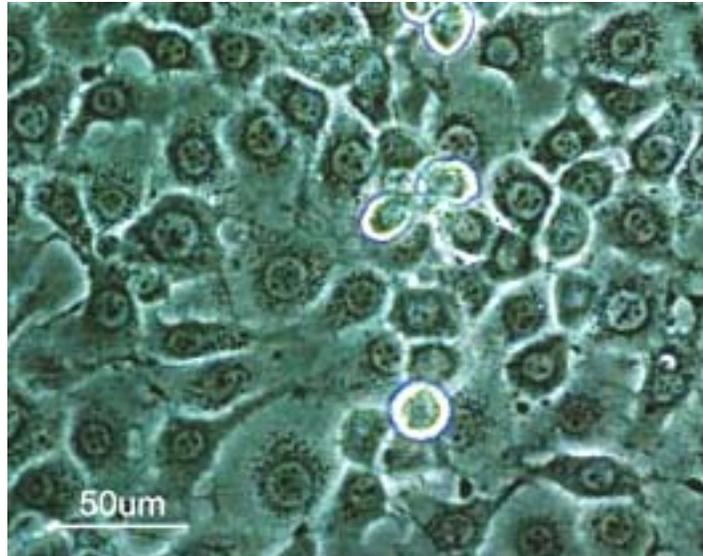
Cell line designation: IPLB-HVT1

Tissue source: *Heliothis virescens* fifth instar larval testes

Date initiated: April 8, 1985

Morphology: epithelial-like, strongly attached cells

Culture medium: Ex-Cell 420 medium (JRH Biosciences) supplemented with 5% (v/v) heat-inactivated fetal bovine serum.



Alternative medium: Modified Grace's medium (TNM-FH) that contains:

Grace's medium	90 ml
lactalbumin hydrolysate	0.3 g
T.C. yeastolate	0.3 g
fetal bovine serum	10 ml

TNM-FH is available from:

- GIBCO (Grace's Insect Cell Culture Medium, Supplemented = cat. # 11605-011)
- SIGMA (TNM-FH Insect Medium = cat. # T3285)
- JRH Biosciences (Hink's TNM-FH Insect Medium = cat. # 51-94278)

Subculture procedure: At one-week intervals, a confluent culture is split by trypsinization. Remove the medium from the culture (save in a sterile test tube) and rinse the cells with buffer (see next page)(use 2 ml buffer to rinse a 25-cm² flask). The rinse is discarded and 1.0 ml VMF Trypsin (0.05 mg/ml of the same buffer) is added. Tilt the flask to be sure entire monolayer is coated with solution and remove (and discard) all but 0.3 ml. Leave at room temperature for 20 min. tilting at 3 to 4 min intervals to make sure the monolayer does not dry out. Gently tap the culture on the bench at 3 to 4 minute intervals to see if the cells have detached, tilting to coat the surface with the buffer after each tap. When they have become loose, add 5 ml fresh medium and suspend cells by repeated pipetting over the flask surface. Transfer 0.3 ml of the cell suspension to a new flask containing 3.7 ml fresh medium. Cells are maintained at 26°C.

Virus susceptibility: Cells are susceptible (with complete occlusion body formation) to: *Autographa californica* NPV and *Amsacta moorei* entomopox virus.

Comments: Morphology suggests these cells originated from the testicular sheath.

Reference: Lynn, D. E., E. M. Dougherty, J. T. McClintock and M. Loeb. Development of cell lines from various tissues of Lepidoptera. *In Invertebrate and Fish Tissue Culture*. Y. Kuroda, E. Kurstak, and K. Maramorosch (eds.) Japan Scientific Societies Press, Tokyo/Springer-Verlag, Berlin, 1988.

Buffer for trypsinization

NaCl	800 mg
KH ₂ PO ₄	20 mg
KCl	20 mg
Na ₂ HPO ₄ ×7H ₂ O	150 mg
Na ₂ EDTA	23 mg

in water to 100 ml

Adjust osmotic pressure with 15% NaCl to 350 mOsm/kg and pH to 7.0 with 2N NaOH. Filter sterilized through 0.2 mm filter and store at 4°C.