Data Sheet

BCRJ Code:	0345
Cell Line:	EA.hy926
Species:	Homo sapiens
Vulgar Name:	Human
Tissue:	Somatic Cell Hybrid/Umbilical Vein
Cell Type:	Endothelial
Morphology:	Endothelial
Growth Properties:	Adherent
Derivation:	The human umbilical vein cell line, EA.hy926, was established by fusing primary human umbilical vein cells with a thioguanine-resistant clone of A549 by exposure to polyethylene glycol (PEG). Hybrid clones were selected in HAT medium and screened for factor VIII-related antigen.
Products:	Genes Expressed: Factor VIII-related antigen; Homo sapiens
Biosafety:	1
Addtional Info:	Electron photomicrographs demonstrate cytoplasmic distribution of Weibel-Palade bodies and tissue-specific organelles, characteristics of differentiated endothelial cell functions such as angiogenesis, homeostasis/thrombosis, blood pressure and inflammation
Culture Medium:	Dulbecco's Modified Eagle's Medium (DMEM) modified to contain 2 mM L-glutamine, 4500 mg/L glucose and 10% of fetal bovine serum.

Subculturing:	Protocol: Volumes used in this protocol are for 75 sq cm flasks; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes. T-75 flasks are recommended for subculturing this product. Remove and discard culture medium. Briefly rinse the cell layer with PBS without calcium and magnesium to remove all traces of serum which contains trypsin inhibitor. Add 2.0 to 3.0 ml of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until cell layer is dispersed (usually within 5 to 15 minutes). Note: To avoid clumping do not agitate the cells by hitting or shaking the flask while waiting for the cells to detach. Cells that are difficult to detach may be placed at 37C to facilitate dispersal. Add 6.0 to 8.0 ml of complete growth medium and aspirate cells by gently pipetting. Transfer cell suspension to a centrifuge tube and spin at approximately 125 X g for 5 to 10 minutes. Discard supernatant. Resuspend the cell pellet in fresh growth medium. Add appropriate aliquots of the cell suspension to new culture vessels. An inoculum of 2 X 10(3) to 3 X 10(3) viable cells/sq. cm is recommended. Incubate cultures at 37C. Subculture when cell concentration reaches between 8 X 10(4) and 1 X 10(5) cells/sq. cm. NOTE: For more information on enzymatic dissociation and subculturing of cell lines consult Chapter 12 in Culture of Animal Cells, a manual of Basic Technique by R. Ian Freshney, 6th edition, published by Alan R. Liss, N.Y., 2010.
Subculturing Medium Renewal:	Twice a week
Subculturing Subcultivation Ratio:	A seeding density of 2 X 103 to 3 X 103 viable cells/sq. cm should be used when subculturing these cells.
Culture Conditions:	Atmosphere: air, 95%; carbon dioxide (CO2), 5% Temperature: 37°C
Cryopreservation:	95% FBS + 5% DMSO (Dimethyl sulfoxide)

Thawing Frozen Cells:	SAFETY PRECAUTION: Is highly recommend that protective gloves and clothing always be used and a full face mask always be worn when handling frozen vials. It is important to note that some vials leak when submersed in liquid nitrogen and will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase may result in the vessel exploding or blowing off its cap with dangerous force creating flying debris. 1. Thaw the vial by gentle agitation in a 37°C water bath. To reduce the possibility of contamination, keep the Oring and cap out of the water. Thawing should be rapid (approximately 2 minutes). 2. Remove the vial from the water bath as soon as the contents are thawed, and decontaminate by dipping in or spraying with 70% ethanol. All of the operations from this point on should be carried out under strict aseptic conditions. 3. For cells that are sensitive to DMSO is recommended that the cryoprotective agent be removed immediately. Transfer the vial contents to a centrifuge tube containing 9.0 mL complete culture medium and spin at approximately 125 x g for 5 to 7 minutes. 4.Discard the supernatant and Resuspend cell pellet with the recommended complete medium (see the specific batch information for the culture recommended dilution ratio). 5. Incubate the culture in a appropriate atmosphere and temperature (see "Culture Conditions" for this cell line). NOTE: It is important to avoid excessive alkalinity of the medium during recovery of the cells. It is suggested that, prior to the addition of the vial contents, the culture vessel containing the growth medium be placed into the incubator for at least 15 minutes to allow the medium to reach its normal pH (7.0 to 7.6).
References:	Edgell CJ, et al. Permanent cell line expressing human factor VIII-related antigen established by hybridization. Proc. Natl. Acad. Sci. USA 80: 3734-3737, 1983. PubMed: 6407019 Bauer J, et al. In vitro model of angiogenesis using a human endothelium-derived permanent cell line: contributions of induced gene expression, G-proteins, and integrins. J. Cell. Physiol. 153: 437-449, 1992. PubMed: 1280276 Edgell CJ, et al. Endothelium specific Weibel-Palade bodies in a continuous human cell line, EA.hy926. In Vitro Cell. Dev. Biol. 26: 1167-1172, 1990. PubMed: 2079463 Rieber AJ, et al. Extent of differentiated gene expression in the human endothelium-derived EA.hy926 cell line. Thromb. Haemostasis 69: 476-480, 1993. PubMed: 8322270
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