Data Sheet

BCRJ Code:	0334
Cell Line:	Fcwf-4 [Fcwf]
Species:	Felis catus
Vulgar Name:	Cat
Tissue:	Fetus, whole
Cell Type:	Macrophage
Morphology:	Spindle to stellate
Disease:	Peritonitis
Growth Properties:	Adherent
Age/Ethinicity:	Fetus /
Applications:	It is used to propagate tissue culture adapted Feline coronavirus (Feline infectious peritonitis virus, FIPV).
Virus Succeptility::	Feline infectious peritonitis virus
Biosafety:	1
Addtional Info:	The cells possess some characteristics of macrophages (nonspecific esterase, phagocytic activity, Fc receptors).
Culture Medium:	Dulbecco's Modified Eagle's Medium (DMEM) with 2 mM L-glutamine and 10% of fetal bovine serum.

Subculturing:	Volumes used in this protocol are for 75 cm2 flask; proportionally reduce or increase amount of dissociation medium for culture vessels of other sizes. Remove and discard culture medium. Briefly rinse the cell layer with PBS without calcium and magnesium to remove all traces of serum that contains trypsin inhibitor. Add 1.0 to 3.0 mL of Trypsin-EDTA solution to flask and observe cells under an inverted microscope until the 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 37°C to facilitate dispersal. Add 6.0 to 8.0 mL of complete growth medium and aspirate cells by gently pipetting. Transfer cell suspension to centrifuge tube and spin at approximately 125 x g for 5 to 10 minutes. Discard supernatant and resuspend cells in fresh growth medium. Add appropriate aliquots of cell suspension to new culture vessels. An inoculum of 1 x 10e4 to 1 x 10e5 viable cells/cm2 is recommended. Do not exceed 1 x 10e6 cells/cm2. Place culture vessels in incubators at 37°C. Interval: Maintain cultures at a cell concentration between 2 x 10e4 and 1 x 10e5 cells/cm2. Population Doubling Time: 31 hrs 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:	2 to 3 times per week
Subculturing Subcultivation Ratio:	1:4 to 1:6
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:	Jacobse-Geels HE, Horzinek MC. Expression of feline infectious peritonitis coronavirus antigens on the surface of feline macrophage-like cells. J. Gen. Virol. 64: 1859-2866, 1983. PubMed: 6886678 Pedersen NC, et al. Infection studies in kittens, using feline infectious peritonitis virus propagated in cell culture. Am. J. Vet. Res. 42: 363-367, 1981. PubMed: 6267959
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