

Data Sheet

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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/Ethnicity:	Fetus /
Applications:	It is used to propagate tissue culture adapted Feline coronavirus (Feline infectious peritonitis virus, FIPV).
Virus Susceptibility::	Feline infectious peritonitis virus
Biosafety:	1
Additional 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.

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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 10⁴ to 1 x 10⁵ viable cells/cm² is recommended. Do not exceed 1 x 10⁶ cells/cm². Place culture vessels in incubators at 37°C. Interval: Maintain cultures at a cell concentration between 2 x 10⁴ and 1 x 10⁵ 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)

SAFETY PRECAUTION: It is strongly recommended to always wear protective gloves, clothing, and a full-face mask when handling frozen vials. Some vials may leak when submerged in liquid nitrogen, allowing nitrogen to slowly enter the vial. Upon thawing, the conversion of liquid nitrogen back to its gas phase may cause the vial to explode or eject its cap with significant force, creating flying debris.

1. Thaw the vial by gently agitating it in a 37°C water bath. To minimize contamination, keep the O-ring and cap out of the water. Thawing should be rapid (approximately 2 minutes).
2. Remove the vial from the water bath as soon as its contents are thawed and decontaminate it by dipping in or spraying with 70% ethanol. From this point, all operations must be performed under strict aseptic conditions.
3. For cells sensitive to DMSO, it is recommended to remove the cryoprotective agent immediately. Transfer the vial contents to a centrifuge tube containing 9.0 mL of complete culture medium and centrifuge at approximately 125 × g for 5 to 7 minutes.
4. Discard the supernatant and resuspend the cell pellet in the recommended complete medium (see specific batch information for the appropriate dilution ratio).
5. Incubate the culture under appropriate atmospheric and temperature conditions (see "Culture Conditions" for this cell line).

NOTE: It is important to avoid excessive alkalinity of the medium during cell recovery. To minimize this risk, it is recommended to place the culture vessel containing the growth medium in the incubator for at least 15 minutes before adding the vial contents. This allows the medium to stabilize at its normal pH (7.0 to 7.6).

Thawing Frozen Cells:

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

Depositors:

Aline Fagundes - Fundação Oswaldo Cruz

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