Data Sheet

BCRJ Code:	0368
Cell Line:	A-72
Species:	Canis familiaris
Vulgar Name:	Dog
Tissue:	Unknown
Cell Type:	Fibroblast
Morphology:	Fibroblast
Disease:	Tumor
Growth Properties:	Adherent
Sex:	Female
Age/Ethinicity:	8 Year /
Derivation:	The line was established from a 1 cm diameter tumor taken from the left thigh of a female Golden Retriever.
Virus Succeptility::	Canine adenovirus 2 Canine coronavirus , Canine coronavirus Canine parainfluenza virus Canid herpesvirus 1 Canine distemper virus Canine minute virus
Biosafety:	1
Culture Medium:	Leibovitz's L-15 Medium contains 2 mM L-glutamine and no sodium bicarbonate and fetal bovine serum to a final concentration of 10%. Note: The L-15 medium formulation was devised for use in a free gas exchange with atmospheric air. A CO2 and air mixture is detrimental to cells when using this medium for cultivation.

Subculturing:	Remove medium, rinse with fresh 0.25% trypsin, 0.53 mM EDTA solution, remove trypsin and let the culture sit at 37°C for 10 to 15 minutes. Add fresh medium, aspirate and dispense into new flasks. 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:	1:2 to 1:4
Culture Conditions:	Atmosphere: air, 100% Temperature: 37°C
Cryopreservation:	95% FBS + 5% DMSO (Dimethyl sulfoxide)

Thawing Frozen Cells:	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).
References:	Binn LN, et al. Establishment of a canine cell line: derivation, characterization, and viral spectrum. Am. J. Vet. Res. 41: 855-860, 1980. PubMed: 6254399 The line was established from a 1 cm diameter tumor taken from the left thigh of a female Golden Retriever. Standard Quantitative Disk Carrier Test Method for Determining the Bactericidal, Virucidal, Fungicidal, Mycobactericidal and Sporicidal Activities of Liquid Chemical Germicides. West Conshohocken, PA:ASTM International;ASTM Standard Test Method E 2197-02.
Depositors:	Ouro Fino Saúde Animal LTDA
Cellosaurus:	CVCL_3453