BCRJ Code:	0400
Cell Line:	CCD-18Co
Species:	Homo sapiens
Vulgar Name:	Human
Tissue:	Colon
Morphology:	Fibroblast
Disease:	Normal
Growth Properties:	Adherent
Sex:	Female
Age/Ethinicity:	2 Month / Black
Biosafety:	1
Addtional Info:	The line begins to senesce at about PDL = 42.
Culture Medium:	DMEM Low glucose + fetal bovine serum to a final concentration of 10%.
Subculturing:	Remove medium, and rinse with 0.25% trypsin, 0.053 mM EDTA solution. Remove the solution and add an additional 1 to 2 mL of trypsin-EDTA solution. Allow the flask to sit at room temperature (or at 37°C) until the cells detach. Add fresh culture medium, aspirate and dispense into new culture flasks. Note: Growth of the cells is enhanced by addition of tumor necrosis factor alpha (TNF alpha) to the medium.
Subculturing Medium Renewal:	Every 2 to 3 days

Subculturing Subcultivation Ratio:	1:2 to 1:3
Culture Conditions:	Atmosphere: air, 95%; carbon dioxide (CO_2), 5% 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:	Sugarman BJ, et al. Recombinant human tumor necrosis factor-alpha: effects on proliferation of normal and transformed cells in vitro. Science 230: 943-945, 1985. PubMed: 3933111 Hinterleitner TA, et al. Il-1 stimulates intestinal myofibroblast COX gene expression and augments activation of Cl- secretion in T84 cells. Am. J. Physiol. 271: C1262-C1268, 1996. PubMed: 8897833

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