Data Sheet

BCRJ Code:	0220
Cell Line:	SF 181
Species:	Homo sapiens
Vulgar Name:	Human
Tissue:	Skin
Cell Type:	Fibroblast
Morphology:	Fibroblast
Disease:	Epidermolysis Bullosa
Growth Properties:	Adherent
Sex:	Male
Age/Ethinicity:	10 Year / Caucasian
Derivation:	Human primary cell line isolated from skin biopsies. Isolated from apatient with epidermolysis bullosa.
DNA Profile:	Amelogenin: X,Y CSF1PO: 10,11 D13S317: 11,12 D16S539: 13 D5S818: 10,13 D7S820: 10,11 THO1: 6,9 TPOX: 11,8 vWA: 16 D2S1338: 19,20 D19S433: 14 FGA: 20,24 D3S1358: 15 D18S51: 13 D8S1179: 13 D21S11: 31,28
Biosafety:	1
Addtional Info:	Formation of blisters and cutaneous lesions upon light mechanical trauma. The byopsia was taken from one of this lesions. Digestive tract also involved, with severe impairement of uper digestive tract function. Patient followed by Dr. Flavio Fraga and Dr. Gisele Pires, Ambulatorio de Imunologia, Hospital Universitario, UFRJ, Rio de Janeiro. Migration assays on collagen gels show a normal phenotype.

Culture Medium:	Dulbecco's Modified Eagle's Medium (DMEM) modified to contain 4 mM L-glutamine, 4500 mg/L glucose 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 medium, and rinse with PBS without calcium and magnesium. 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: 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
Culture Conditions:	Atmosphere: air, 95%; carbon dioxide (CO2), 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).
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Cellosaurus:	CVCL_D682