コスモ・バイオ株式会社
CellResearchCorp

Product Information

HIGH QUALITY LOW PASSAGE PRIMARY SKIN CELL CULTURES

Fibroblasts, Keratinocytes and Melanocytes derived from skin and scar/keloid tissue (*various anatomical regions available; juvenile, adult and aging*), provided in T75 cultured flasks. 2ml cryopreserved vials are also available.
> 500,000 viable or proliferating cells

Product Line

- ❑ **N**ormal Human Dermal **F**ibroblasts **(NF)**
- ❑ **K**eloid **F**ibroblasts **(KF)**
- ❑ **H**ypertrophic **S**car-derived **F**ibroblasts **(HSF)**
- ❑ **F**ibroblast isolated from **N**ormal **S**car **(NSCF)**
- ❑ Fibroblasts isolated from **n**ormal **s**kin adjacent to **K**eloid **F**ibroblasts **(nsKF)** or **H**ypertrophic **S**cars **(nsHSF)**
- ❑ **F**ibroblasts isolated from **A**ging **S**kin **(asF)**
- ❑ **N**ormal Human **K**eratinocytes **(NK)**
- ❑ **K**eloid-derived **K**eratinocytes **(KK)**
- ❑ **H**ypertrophic **S**car-derived **K**eratinocytes **(HSK)**
- ❑ **K**eratinocytes isolated from **N**ormal **S**car **(NSCK)**
- ❑ **K**eratinocytes isolated from **n**ormal **s**kin adjacent to **K**eloid **F**ibroblasts **(nsKK)** or **H**ypertrophic **S**cars **(nsHSK)**

Cell Culture Description

Using Explant Technique as the primary cell culture method, the high quality low passage cell strains are derived at CellResearch's cell culture facility from skin and scar/keloid tissue obtained from surgical procedures. Each strain is obtained from one individual and isolated according to referenced procedures. Proliferating cell cultures are made from cryopreserved cells that have been thawed and cultured for three days at CellResearch's cell culture facility. Cells are not pooled or transformed.

Proliferating Capacity

CellResearch's cell cultures are derived with the use of careful methods, from skin and scar/keloid tissue (in vivo state). They are not transformed and have a limited lifespan in vitro. All strains are tested for their proliferative capacity in CellResearch's cell culture facility.

Quality Test

All cell cultures from CellResearch are subjected to stringent quality tests before shipment. Comprehensive testing include HIV-1 PCR, HBV DNA PCR and HCV RNA PCR. Certificate of Analysis (CoA) will accompany shipments.

Maintenance of Cryoperserved cells

Upon receipt of delivery, the vials with the cryopreserved cells must be taken out of the dry ice container immediately and;

a. be transferred to a storage facility with liquid nitrogen (-196°C), or
b. thawed and put each vial in a T75 tissue culture flask in DMEM/10%FCS (Fibroblast) or Serum-Free Medium (Keratinocyte)

Maintenance of Proliferating cells

Upon receipt of delivery,

a. Check the proliferating culture for signs of damage during dispatch (e.g. atypical morphology). The bottles should show many cell "islands". Determine the cell density by estimating the "confluence %".
b. Place the closed culture flask in a 37°C, humidified incubator with 5% CO2.
c. Prepare medium.
d. Wipe the culture flask with 70% ethanol and wait until the alcohol has evaporated before opening the culture flask in a laminar airflow cabinet. Remove the medium with a pipette without touching the cell monolayer. Replace the medium with 10 ml fresh medium. In order to prevent contamination make sure that there are no traces of medium left on the inner / outer part of the neck of the culture flask.
e. Place the filled cell culture flask in a 37°C, humidified incubator with 5% CO2. Close the screw lids on the culture flask by half a turn only to allow gas exchanges to take place.
f. The cells are ready for sub-culturing after 12 to 48 hours.

Warning Note (Use of biological material)

CellResearch's cell cultures are of human origin and while every cryopreserved cells have been tested as per our quality test, no diagnostics tests can ensure the total absence of infectious agents. All cells of human origin should be treated as potential pathogens.

FOR RESEARCH USE ONLY. NOT TO BE USED FOR DIAGNOSTICS OR THERAPEUTIC PURPOSES.

Ordering and Technical Information
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CellResearchCorp

UEN: 200208514E

Donor	Product Code	Region
	Normal Fibroblast	
F, C, 46yr	NF101	Breast
M, C, 8m	NF103	Right duplicate thumb
F, M, 14m	NF104	Groin
M, C, 21yr	NF105	Lateral arm
F, E, 47yr	NF106	Right breast
M, M, 23yr	NF107L	Left Axillary Skin
M, M, 23yr	NF107R	Right Axillary Skin
M, C, 39yr	NF108	Left hand dorsum
M, I, 39yr	NF109	Left forearm
F, C, 23yr	NF110	Eyelid Skin
M, C, 29yr	NF111	Left forearm volar
M, I, 23yr	NF113	Right wrist
M, M, 50yr	NF114	Right thigh
F, A, 10yr	NF115	Right abdominal Scar
F, I, 31yr	NF116	Earring Clelts
F, C, 52yr	NF117	Forehead
F, I, 35yr	NF118	Abdominoplasty
M, A, 37yr	NF119	R Back (SD)
F, A, 43yr	NF120	Bilateral earlobe
M, C, 54yr	NF121	Eyelid skin
F, F, 43yr	NF122	Bilateral Eyelid skin
F, C, 48yr	NF123	Right Cheek (SD)
F, C, 35yr	NF124	Upper Eyelid skin
F, C, 54yr	NF125	Upper Lip
F, C, 42yr	NF126	Rt Forehd Adj vas malf
F, C, 27yr	NF127	Back skin
F, A, 44yr	NF128	Abdominoplasty
M, A, 51yr	NF129	Right temple skin
F, A, 40yr	NF130	Abdominoplasty
M, A, 52yr	NF131	Abdominoplasty (SD)
F, I, 42yr	NF132	Rt Cleft earlobe
M, C, 37yr	NF133	Rt Calf Skin (SD)
M, C, 32yr	NF134	Skin adj to dermatolibroma (SD)
M, C, 36yr	NF135	Skin adj to dermatolibroma
M, E, 40yr	NF136	Peridermal naevus (SD)
F, C, 44yr	NF137	Abdominoplasty
F, C, 40yr	NF138	Abdominoplasty
F, C, 40yr	NF139	Supra-Eyebrow
F, A, 38yr	NF140	Rt Traumatic Earlobe Cleft
F, A, 36yr	NF141	Abdominoplasty
F, C, 40yr	NF142	Eyelid skin
F, C, 46yr	NF143	Eyelid skin
F, A, 39yr	NF144	Radial Abdominoplasty
F, I, 45yr	NF145	Cleft earlobe
M, A, 50yr	NF146	Rt forehead
F, A, 38yr	NF147	Abdominoplasty
M, A, 38yr	NF148	Left Earlobe Skin
F, A, 46yr	NF149	Labioplasty
F, A, 33yr	NF150	Abdominoplasty
F, C, 43yr	NF151	Earlobe
F, C, 43yr	NF152	Eyelid skin
F, M, 53yr	NF153	Eyelid skin
F, A, 46yr	NF154	Earlobe skin
F, I, 24yr	NF155	Rt Cleft earlobe
F, A, 38yr	NF156	Eyelid skin
F, C, 49yr	NF158	Upper eyelid skin
F, C, 22yr	NF159	Alar skin
F, V, 29yr	NF160	Forehead
F, A, 44yr	NF161	Bila Upp Eyelid skin
F, C, 50yr	NF162	Upper Eyelid skin
F, C, 34yr	NF164	Abdominoplasty
F, A, 42yr	NF165	Bila Cleft Earlobe skin
F, N, 46yr	NF166	Breast
F, A, 45yr	NF167	Abdominoplasty
F, C, 29yr	NF168	Abdominoplasty
F, C, 46yr	NF169	Eyelid Skin
M, A, 51yr	NF170	Rt Wrist Skin
M, A, 52yr	NF171	Ear
M, A, 51yr	NF172	Eyelid Skin
F, I, 45yr	NF173	Breast
F, I, 35yr	NF174	Abdominoplastry
F, C, 13yr	NF175	Ulnar Forearm (Flexor Surface)
F, C, 46yr	NF176	Abdominoplastry
M, V, 37yr	hPDF100	Peridontal
M, V, 37yr	hOMF100	Oral Mucosa
F, V, 26yr	hOMF101	Lip mucosa (from rev)
M, M, 58yr	hOMF102	Oral Mucosa (Gingivia)
F, C, 52yr	hOMF107	Oral Mucosa (Gum)
F, A, 57yr	hOMF108	Oral Mucosa (Gum)
M, C, 66yr	hOMF109	Oral Mucosa (Gum)
M, C, 61yr	asF1	Right groin
F, M, 59yr	asF2	Right chest

Legend
Order: Gender, Race, Age

F: Female
M: Male

A: Caucasian
C: Chinese
E: Eurasian
F: Filipino
I: Indian
M: Malay
N: Nigerian
V: Vietnamese/Cambodian

yr: Years
m: Months

SD: Sun Damaged

Product Code	Region
Normal Keratinocyte	
NK101	Breast
NK103	Right duplicate thumb
NK104	Groin
NK105	Lateral arm
NK106	Right breast
NK107	Axillary Skin
NK108	Left hand dorsum
NK109	Left forearm
NK110	Eyelid Skin
NK111	Left forearm volar
NK113	Right wrist
NK114	Right thigh
NK116	Earring Clelts
NK117	Forehead
NK118	Abdominoplasty
NK120	Bilateral earlobe
NK121	Eyelid skin
NK122	Bilateral Eyelid skin
NK123	Right Cheek (SD)
NK124	Upper Eyelid skin
NK125	Upper Lip
NK126	Rt Forehd Adj vas malf
NK127	Back skin
NK128	Abdominoplasty
NK129	Right temple skin
NK130	Abdominoplasty
NK131	Abdominoplasty (SD)
NK132	Rt Cleft earlobe
NK133	Rt Calf Skin (SD)
NK134	Skin adj to dermatolibroma (SD)
NK135	Skin adj to dermatolibroma
NK136	Peridermal naevus (SD)
NK137	Abdominoplasty
NK138	Abdominoplasty
NK139	Supra-Eyebrow
NK140	Rt Traumatic Earlobe Cleft
NK141	Abdominoplasty
NK142	Eyelid skin
NK143	Eyelid skin
NK144	Radial Abdominoplasty
NK145	Cleft earlobe
NK146	Rt forehead
NK147	Abdominoplasty
NK148	Left Earlobe Skin
NK149	Labioplasty
NK150	Abdominoplasty
NK151	Earlobe
NK152	Eyelid skin
NK153	Eyelid skin
NK154	Earlobe skin
NK155	Rt Cleft earlobe
NK156	Eyelid skin
NK158	Upper eyelid skin
NK159	Alar skin
NK160	Forehead
NK161	Bila Upp Eyelid skin
NK162	Upper Eyelid skin
NK164	Abdominoplasty
NK165	Bila Cleft Earlobe skin
NK166	Breast
NK167	Abdominoplasty
NK168	Abdominoplasty
NK169	Eyelid Skin
NK170	Rt Wrist Skin
NK171	Ear
NK172	Eyelid skin
NK173	Breast
NK174	Abdominoplastry
NK175	Ulnar Forearm (Flexor Surface)
NK176	Abdominoplastry
hOMK100	Oral Mucosa
hOMK101	Lip mucosa (from rev)
hOMK102	Oral Mucosa (Gingivia)
hOMK107	Oral Mucosa (Gum)
hOMK108	Oral Mucosa (Gum)
hOMK109	Oral Mucosa (Gum)

F, C, 84yr	asF3	Left calf
M, C, 52yr	asF4	Right thigh
M, C, 78yr	asF5	Back neck
F, C, 62yr	asF6	Bilateral upper eyelid
M, C, 60yr	asF7	Back
M, A, 60yr	asF8	L Preauricular (SD)
F, C, 58yr	asF9	Hand (Radial / Aluar)
F, C, 60yr	asF10	Eyelid
F, C, 59yr	asF11	Lt Forearm Skin (SD)
M, A, 61yr	asF12	Upper Abdomen
F, A, 56yr	asF13	Back skin adj to SD skin
F, C, 56yr	asF14	Facelift skin
F, C, 57yr	asF15	Upper Eyelid skin
F, C, 56yr	asF16	Facelift skin
F, A, 55yr	asF17	Abdominoplasty
F, A, 59yr	asF18	Abdominoplasty
F, C, 59yr	asF19	Eyelid skin
F, C, 69yr	asF20	Eyelid skin
M, A, 55yr	asF21 L/R	Cheek (Face)
M, A, 55yr	asF22 L/R	Neck
M, A, 56yr	asF23	Upper Eyelid skin
F, C, 70yr	asF24	Preocular (Ear)
F, C, 69yr	asF25	Lower Eyelid Skin
F, C, 90yr	asF26	Rt Forearm skin
F, A, 56yr	asF27	Abdominoplasty
F, A, 65yr	asF28	Abdominoplasty
M, C, 66yr	asF29	Bilateral Eyelid skin
F, C, 77yr	asF30	Upper Eyelid skin
M, C, 76yr	asF31	Rt Medial Heel Skin (lesion)
F, C, 57yr	asF32	Upper Eyelid skin
M, C, 67yr	asF33	Upper Eyelid skin

	asK5	Back neck
	asK6	Bilateral upper eyelid
	asK7	Back
	asK8	
	asK9	Hand (Radial / Aluar)
	asK10	Eyelid
	asK11	Lt Forearm Skin (SD)
	asK12	Upper Abdomen
	asK14	Facelift skin
	asK16	Facelift skin
	asK17	Abdominoplasty
	asK18	Abdominoplasty
	asK19	Eyelid skin
	asK20	Eyelid skin
	asK21 L/R	Cheek (Face)
	asK22 L/R	Neck
	asK23	Upper Eyelid skin
	asK24	Preocular (Ear)
	asK25	Lower Eyelid Skin
	asK26	Rt Forearm skin
	asK27	Abdominoplasty
	asK28	Abdominoplasty
	asK29	Bilateral Eyelid skin
	asK30	Upper Eyelid skin
	asK31	Rt Medial Heel Skin (lesion)
	asK32	Upper Eyelid skin
	asK33	Upper Eyelid skin

	Melanocytes	
M, I, 23yr	HM-1	Right wrist
M, M, 50yr	HM-2	Right thigh

	Keloid Fibroblast		**Fibroblast adj to NS of Keloid**		**Keloid-derived Keratinocyte**	
M, C, 27yr	KF101	Right wrist				
F, C, 17yr	KF103	Left earlobe				
F, C, 35yr	KF104	Left deltoid	nsKF104	Left deltoid		
F, M, 12yr	KF105	Right helix ear				
F, C, 22yr	KF106	Left earlobe				
M, C, 23yr	KF107	Left anterior earlobe				
M, C, 23yr	KF108	Left posterior earlobe				
M, C, 27yr	KF109	Right elbow			KK109	Right elbow
M, M, 9yr	KF110	Left cheek	nsKF110	Left cheek	KK110	Left cheek
F, C, 21yr	KF111	Right back	nsKF111	Right back	KK111*	Right back
M, C, 35yr	KF112	Right cheek	nsKF112	Right cheek	KK112*	Right cheek
F, C, 22yr	KF113	Left earlobe (Helix)			KK113	Left earlobe (Helix)
F, M, 17yr	KF114	Right back	nsKF114	Right back	KK114*	Right back
F, C, 37yr	KF115	Rt Calf Underspread			KK115	Rt Calf Underspread
F, I, 47yr	KF116 L/R	Earlobe			KK116 L/R	Earlobe
M, C, 31yr	KF117	Left Earlobe			KK117	Left Earlobe
F, C, 43yr	KF118	Pfannenstiel incision	nsKF118	Pfannenstiel incision	KK118*	Pfannenstiel incision
M, C, 32yr	KF119	Left Ear	nsKF119	Left Ear	KK119*	Left Ear

	Hypertrophic Scar Fibroblast		**Fibroblast adj to NS of H-Scar**		**H-Scar derived Keratinocyte**	
F, C, 29yr	HSF101	Left wrist				
M, C, 23yr	HSF102	Right hand			HSK102	Right hand
F, C, 29yr	HSF103	Left arm				
M, C, 21yr	HSF104	Right foot				
M, I, 28yr	HSF105	Left hand	nsHSF105	Left hand		
F, C, 28yr	HSF106	Right forearm	nsHSF106	Right forearm		
M, N, 51yr	HSF107	Radial forearm	nsHSF107	Radial forearm	HSK107	Radial forearm
F, A, 38yr	HSF108	Abdominoplasty	nsHSF108	Abdominoplasty	HSK108	Abdominoplasty
F, C, 27yr	HSF109	Rt Buttocks	nsHSF109	Rt Buttocks	HSK109	Rt Buttocks
M, M, 42yr	HSF110	Lt Cheek	nsHSF110	Lt Cheek	HSK110	Lt Cheek
M, C, 12yr	HSF111	Lt Panietal Scalp Scar	nsHSF111	Lt Panietal Scalp Scar	HSK111	Lt Panietal Scalp Scar
F, V, 24yr	HSF112	Lt Shin Scar	nsHSF112	Lt Shin Scar	HSK112	Lt Shin Scar
F, V, 29yr	HSF113	Lt Calf Scar	nsHSF113	Lt Calf Scar	HSK113	Lt Calf Scar
M, V, 16yr	HSF114	Rt Hand	nsHSF114	Rt Hand	HSK114	Rt Hand

	Normal Scar Fibroblast		**N-Scar derived Keratinocyte**	
M, C, 39yr	NSCF1	Left hand dorsum	NSCK1	Left hand dorsum
F, V, 18yr	NSCF2	Left Cleft Lip Sear	NSCK2	Left Cleft Lip Sear
F, A, 38yr	NSCF3	Abdominoplasty	NSCK3	Abdominoplasty
F, A, 33yr	NSCF4	Abdominoplasty	NSCK4	Abdominoplasty
F, C, 35yr	NSCF5	Lt Cheek Facial Scar	NSCK5	Lt Cheek Facial Scar
F, C, 36yr	NSCF6	Lt Jawline Depressed Scar	NSCK6	Lt Jawline Depressed Scar
F, V, 33yr	NSCF7	Rt Knee Scar	NSCK7	Rt Knee Scar
F, C, 32yr	NSCF8	Bilateral Breast Scar	NSCK8	Bilateral Breast Scar
M, C, 32yr	NSCF9	Lt Chest Scar	NSCK9	Lt Chest Scar
F, C, 17yr	NSCF10	Lt Knee Scar	NSCK10	Lt Knee Scar
F, A, 34yr	NSCF11	Lt Leg Scar	NSCK11	Lt Leg Scar
M, C, 19yr	NSCF12	Rt Abdomen Scar	NSCK12	Rt Abdomen Scar
F, C, 40yr	NSCF13	Abdominoplasty	NSCK13	Abdominoplasty

* Keratinocytes isolated from normal skin adjacent to Keloid

Note: For specific site regions or other cell types, please check with us or our distributors

9-Apr-13

CellResearchCorp

Co. Reg 200208514E

Human Dermal Fibroblast Cell Systems

Instructions for Use

Unpacking and Storage Instructions

1. Check all containers for leakage or breakage.

2. For cryopreserved cells – If there is dry ice left in the package, place cryovials immediately into liquid nitrogen. If no dry ice is left in the package, thaw and use them immediately.

3. For proliferating cells – Swab down the flask of proliferating cells with 70% ethanol or isopropanol, then place the flask in 37°C, 5% CO2, humidified incubator and allow equilibrating for three to four hours. After cells have equilibrated, remove shipping medium from the flask and replace with fresh medium.

Cell Culture Medium and Reagents:

DMEM supplemented with 10% Fetal Bovine Serum and 1% Antibiotics/Antimycotics

1x Trypsin-EDTA solution (from Trypsin-EDTA (10X), 0.5% Trypsin with EDTA-4Na Cat#15400-055, Invitrogen Corporation)

Cell Culture Process

1. The recommended seeding density for Human Dermal Fibroblasts is 3,300 cells/cm^2 or 250,000 cells/T75 tissue culture flask. Cells will reach 100% confluent in 5-7 days.

2. Wipe cryovial with ethanol or isopropanol before opening. In a sterile field, briefly twist the cap a quarter turn to relieve pressure, and then retighten.

3. Quickly thaw the cryovial in a 37°C water bath being careful not to submerge the entire vial.

4. Re-suspend the cells in the cryovial and using a micropipette, dispense cells into the culture vessels set up earlier. Gently rock the culture vessel to evenly distribute the cells and return to the incubator.

5. Centrifugation should not be performed to remove cells from cryoprotectant cocktail. This action is more damaging than the effects of DMSO residue in the culture.

Sub-culture of Human Dermal Fibroblasts

The following instructions are for a 75 cm^2 flask. Adjust all volumes accordingly for other size flasks.

Preparation for subculturing the first flask:

1. Subculture the cells when they are 80% to 90% confluent and contain many mitotic figures throughout the flask.

2. For each 75 cm^2 of cells to be subcultured:

   Add 5 ml of warm 1X Trypsin/EDTA in each flask.

   Incubate in incubator for less than 5min.

   Shake gently to detach cells from bottom of the flasks.

   Transfer to centrifuge tube.

   Pipette up and down vigorously to break cell clump.

   Add in the tube 4ml DMEM/10%FCS to quench trypsin action.

   Centrifuge at 1200 rpm for 5 min.

   Suspend cell pellet in DMEM/10%FCS for further experiment or cryopreservation

3. Subculture 1-3 flasks at a time.

4. Cryo-preserve cells in DMEM/10%FCS/10%DMSO. Recommend cell density of 500,000-2,000,000 cells/2ml cryovial.

Frequently Asked Questions (FAQ)

Technical

1. Can I freeze CellResearch's cell strains?

It is not advisable to freeze the cell strains as this may lead to a degradation of the cells and their proliferating potential.

2. What techniques are you using to isolate the cell strains?

Explant Technique is used in the isolation of the cell strains.

3. Are CellResearch's cells strains derived from a single individual or are they pooled from several donors?

CellResearch's cell strains are derived from single donors and are not pooled.

4. Which anatomical regions are the cell strains derived from?

We have cell strains from various anatomical regions such as earlobe, groin, wrist, leg, etc. Please consult with our sales specialists.

5. Are the Keloid fibroblasts considered cancerous?

The Keloid fibroblasts are not cancerous, but may be considered benign tumor. This is because there are some cancerous characteristics, such as fast proliferation, invasiveness, fast migration, apoptotic genes are down regulated and anti-apoptotic genes are up regulated, etc.

6. Which cells produce more collagen?

Keloid fibroblasts produce approximately 20 times more collagen than normal dermal fibroblasts while aging fibroblasts produce less collagen than young skin fibroblasts.

7. Which cells are more elastic?

Keloid and Hypertrophic Scar cells are less elastic than normal skin cells.

8. Are the cells tested against potential infectious hazards?

The cells are analyzed for HIV, HBV and HCV. However, note that CellResearch's cell cultures are of human origin and while every effort has been taken to test the cells, no diagnostics tests can ensure the total absence of infectious agents. All cells of human origin should be treated as potential pathogens.

General

1. Why should I use CellResearch's cell strains?

CellResearch's cell strains are obtained from single donors and are not pooled. We have strains derived from multiple locations of the body. Besides the normal human skins, we also have cell strains derived from Keloid and Hypertrophic Scars. For comparative research, fibroblasts isolated from normal skin adjacent to the Keloid or Hypertrophic Scar is also available.

2. How are the cells supplied?

As proliferating cells in CF, unless specified. Cryoperserved vials are also available upon request.

If your questions have not yet been answered, please contact our **Technical Specialists**.

They will be most happy to clarify your queries.