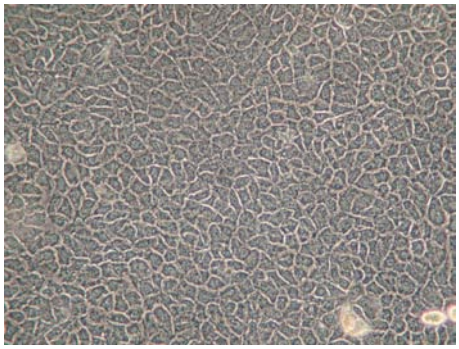
INBIOBANK www.inbiobank.org
Paseo Mikeletegi, 61 bajo. San Sebastian 20009 (Spain)
Phone: +34 943 309064 Fax: +34 943 308222
info@inbiobank.org

PRIMARY HUMAN KERATINOCYTES

Processed directly from adult human skin (from healthy donors) and expanded *in vitro* for a maximum of three passages in the presence of a lethally irradiated 3T3 feeder cell layer, by following the classical procedure of Rheinwald and Green [1, 2]. Cells are supplied in 2 ml cryotubes containing **1 million** cells in 1 ml FBS (v/v) dimethylsulphoxide (DMSO).



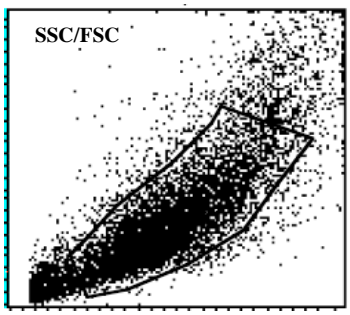
Quality Controls

All cells have been processed in INBIOBANK by manufacturing procedures based on ISO9001:2000 standards. These cells have tested negative for the following pathogens: HIV-1/2, Hepatitis B/C (Abbot Prism Chemiluminiscence Assay) and Mycoplasma (PCR Kit).

Cellular morphology and viability have also been tested after recovery from criopreservation.

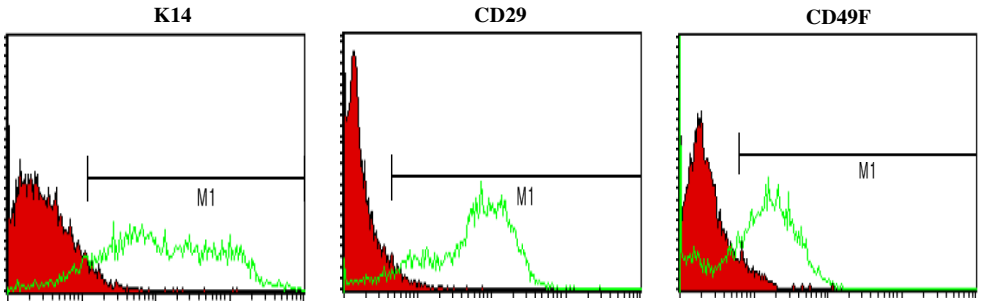
Cell characterisation

Human keratinocytes are characterised morphologically through serial passage, and each batch is tested for expresion of the following keratinocyte markers: cytokeratin 14 (K14), integrin alpha-6 (CD49f), integrin beta-1 (CD29).



S0701K
Age 39
Sex: Female

Red area: Isotypic control
Green line: specific CD



INBIOBANK www.inbiobank.org
Paseo Mikeletegi, 61 bajo. San Sebastian 20009 (Spain)
Phone: +34 943 309064 Fax: +34 943 308222
info@inbiobank.org

Instructions for use

Storage:

- The cells may be supplied criopreserved in dry ice. When received, liquid nitrogen storage is recommended until use.

Cell thawing:

- Transfer to 37ºC water bath.
- Gently agitate the vial while holding the tip with a forceps, being careful not to allow water to penetrate the cap or seal.
- Quickly sterilize vial with 70% ethanol and transfer to a laminar flow hood. Add 10ml warm complete media to a 15ml tube. When completely thawed, slowly transfer contents of vial to 15ml test tube and spin at 300g for 5min.
- Remove and discard the medium and resuspend in fresh culture medium (w/o Epidermal growth factor, EGF).

Plating and passaging of keratinocyte cultures.

- Cells must be seeded simultaneously with lethally irradiated mouse 3T3 cells (Swiss Albino, ECACC #85022108). These cells are irradiated at 50 Gy and stored at 4°C until use for a maximum of 4 days.
- INBIOBANK recommends a plating density of about 25,000 keratinocytes/cm^2 and 90,000 low passage 3T3 cells/cm^2, in medium w/o EGF.
- Do not disturb the plates for the first 48 hours.
- Change the culture medium to medium with EGF, and refresh media every 2-3 days. Feeder layer also needs replenishment every week.
- Expand the cells when 80-90% confluent.

Warning

FOR RESEARCH USE ONLY. Not for use in clinical or veterinary assays, transplants or *in vitro* diagnostic procedures.

Human keratinocyte growth medium	**Working conc.**
Dulbecco's modified Eagle's medium (DMEM, 4,500 mg glucose/l)	60%
Ham's F-12 Nutrient mixture	30%

INBIOBANK www.inbiobank.org
Paseo Mikeletegi, 61 bajo. San Sebastian 20009 (Spain)
Phone: +34 943 309064 Fax: +34 943 308222
info@inbiobank.org

Foetal bovine serum[†]	10%
L-Glutamine	584 mg/l
Penicillin/Streptomycin	50 ug/ml
Adenin	0,18 mM
Cholera toxin	0,1 nM
Thyroid hormone T3	2 nM
Hydrocortisone	0,4 ug/ml
Bovine insulin	5 ug/ml
Epidermal growth factor (EGF)[‡]	10 ng/ml

References

1. Rheinwald, J.G. and H. Green, *Serial cultivation of strains of human epidermal keratinocytes: the formation of keratinizing colonies from single cells*. Cell, 1975. **6**(3): p. 331-43.
2. Rheinwald, J.G. and H. Green, *Epidermal growth factor and the multiplication of cultured human epidermal keratinocytes*. Nature, 1977. **265**(5593): p. 421-4.

[†] End user must test specific serum batches for optimal cell growth.

[‡] Media must be prepared with and w/o EGF.