



IN-VITRO EFFICACY ASSESSMENT OF RAW MATERIALS and FINISHED PRODUCTS

STUDY	TEST PROCEDURE	CELL AND TISSUE MODELS*	STUDY CODE
MOISTURIZING /HOMEOSTASIS			
AQUAPORINS EXPRESSION	Assessment of modulating effects on aquaporins expression ▪ Quantification of Aquaporin-3 content - Elisa assay	NHEK RHEps	AQP3
HYALURONIC ACID PRODUCTION	Assessment of hyaluronic acid production ▪ Quantification of hyaluronic acid content – Elisa assay	NHDF NHEK	SHYAL
HYALURONIDASE ACTIVITY	Assessment of inhibiting effects on hyaluronidase activity ▪ Measurement of Hyaluronidase activity – Enzyme activity assay	NHDF	HYALase
SKIN PERMEABILITY			
BARRIER FUNCTION	Assessment of modulating effects on « Skin barrier function » ▪ SDS or UVB Stress ▪ Measurement of transepithelial permability in basal conditions [(-) stress] and in "altered permeability" condition [with stress] – FITC-tracer flux assay	NHEK (culture units with 2 compartments) RHEps	FBAR-K2 BAR-ER
BIOSTIMULATING ACTIVITY			
CELLULAR GROWTH	Assessment of cellular growth ▪ Measurement of cell density by colorimetric assay - Neutral Red Uptake (NRU) test ▪ Growth curve establishment	NHDF- HaCaT NHEK NHEM	PROL
CELL DIFFERENTIATION			
INVOLUCRIN EXPRESSION	Assessment of Involucrin expression ▪ Measurement of Involucrin content – Elisa assay	NHEK	DIF-INVOL
FILAGGRIN EXPRESSION	Assesment of Filaggrin expression ▪ Measurement of Filaggrin content - Western Blot analysis	NHEK	DIF-FLG
TRANGLUTAMINASE EXPRESSION	Assessment of Transglutaminase expression ▪ Measurement of Transglutaminase content - Western Blot analysis	NHEK	DIF-TGASE
TRANGLUTAMINASE ACTIVITY	Assessment of Transglutaminse activity ▪ Measurement of Transglutaminase type I activity – Colorimetric microassay	NHEK	TGASE
E-CADHERIN EXPRESSION	Assessment of modulating effects on E-Cadherin expression Inducer : Basal/H ₂ O ₂ Evaluation of E-cadherin expression – Elisa assay	NHEK	ECAD

*cf. Appendix p. 11



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ANTI-APOPTOSIS ACTIVITY			
DNA FRAGMENTATION and /or CASPASE ACTIVITY	<p>Assessment of modulating effects on DNA fragmentation induced by UVB</p> <ul style="list-style-type: none"> Quantification of histone-associated DNA fragments (mono- and oligonucleosomes) – Elisa assay <p>Assessment of modulating effects on Caspase-3 activity induced by UVB</p> <ul style="list-style-type: none"> Quantification of Caspase-3 activity – Elisa assay 	NHEK HaCaT RHEPs	APO
ANTI-DNA DAMAGE			
GENOPROTECTIVE ACTIVITY	<p>Assessment of protective effects against DNA damage induced by UVA and UVB</p> <ul style="list-style-type: none"> UVB genoprotective effect: quantification of histone-associated DNA fragments (mono- and oligonucleosomes) – Elisa assay UVA genoprotective effect: quantification of oxidative DNA adduct - 8-Hydroxydesoxyguanosine (8-OHdG) – Elisa assay 	NHEK HaCaT	ADD
DNA REPAIR ACTIVITY	<p>Assessment of modulating effects against UVB-induced DNA-damage and repair</p> <ul style="list-style-type: none"> Kinetic measurement of CPD content (cyclobutane pyrimidine dimers) and/or 6-4PP (pyrimidine (6-4) pyrimidone) photoproducts. 	NHEK HaCaT	REPADN
ANTI-GLYCATION ACTIVITY			
AGEs INDUCED FIBROBLAST APOPTOSIS	<p>Assessment of modulating effects on apoptosis induced by AGEs (Advanced Glycation End products)</p> <p>Inducer : CML-Coll (carboxymethyl-collagen)</p> <ul style="list-style-type: none"> Quantification of histone-associated DNA fragments (mono- and oligonucleosomes) – Elisa assay Quantification of Caspase-3 activity – Elisa assay 	NHDF	AGE-APO
AGE-induced RAGE EXPRESSION	<p>Assessment of modulating effects on RAGE expression</p> <p>Inducer : CML-BSA ou TNF-α</p> <ul style="list-style-type: none"> Quantification of RAGE expression (<i>Receptor for Advanced Glycation End products</i>) - Western Blot 	NHDF	AGE-RAGE
PROTEIN ANTI- GLYCATION ACTIVITY	<p>BSA glycation : assessment of modulating effects on protein glycation</p> <ul style="list-style-type: none"> Study performed in BSA (Bovin serum albumin) incubated with D-glucose. Quantification of AGEs level by measuring fluorescence at days 7,14 and 21 	IN TUBO	AGE-T
	<p>Collagen glycation : assessment of modulating effects on protein glycation</p> <ul style="list-style-type: none"> Study performed in collagen gel incubated with D-Glucose Quantification of AGEs level by measuring fluorescence at days 4-5 	IN TUBO	AGC-T

STUDY	TEST PROCEDURE	CELL AND TISSUE MODELS*	STUDY CODE
DERMAL REGENERATION / EXTRACELLULAR MATRIX			
COLLAGEN I and/or III SYNTHESIS	Assessment of modulating effects on collagen synthesis <ul style="list-style-type: none"> Measurement of collagen type I and /or III levels – Elisa assay 	NHDF	SCOL
PROCOLLAGEN I SYNTHESIS	Assessment of modulating effects on collagen I neosynthesis <ul style="list-style-type: none"> Measurement of Procollagen type I C-peptide – Elisa assay 	NHDF	SCOL-PIP
ELASTIN SYNTHESIS	Assessment of modulating effect on elastin synthesis <ul style="list-style-type: none"> Measurement of elastin level – Elisa Assay 	NHDF	ELAST
FIBRONECTIN SYNTHESIS	Assessment of fibronectin synthesis <ul style="list-style-type: none"> Measurement of fibronectin content – Elisa Assay 	NHDF	SFIB
EXTRACELLULAR MATRIX DEGRADATION	Assessment of modulating effect on MMPs production Inducer : IL-1 β or TNF- α <ul style="list-style-type: none"> Measurement of MMPs (MMP-1 and/or MMP-2) activity in cell culture supernatant - Elisa assay 	NHDF (Dermal equivalent)	MMP
	Assessment of modulating effects on photo-damaged Extracellular Matrix Degradation Inducer : UVA <ul style="list-style-type: none"> Measurement of Procollagen type I C-peptide – Elisa assay Measurement of MMP-1 activity in cell culture supernatant - Elisa assay 	NHDF	DAMEC
	Assessment of modulating effects on fibroblast elastase activity Inducer : UVA <ul style="list-style-type: none"> Measurement of elastase activity – cell-based enzymatic assay 	NHDF	ELAASE
DERMO-EPIDERMAL JUNCTION – BASEMENT MEMBRANE			
COLLAGEN IV AND VII SYNTHESIS	Assessment of modulating effects on collagen IV and VII synthesis <ul style="list-style-type: none"> Measurement of collagen IV and VII levels in incubation medium of skin equivalent – Elisa assay 	Skin equivalent	MB
LAMININ SYNTHESIS	Assessment on modulating effects on Laminin synthesis <ul style="list-style-type: none"> Measurement of laminin level – Elisa Assay 	Skin equivalent	LAM



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ANTIOXIDANT / FREE RADICAL SCAVENGING (#part 1)			
ANTIOXIDANT ACTIVITY	Assessment of Antioxidant potential - Intracellular Oxidative Status Inducer : UVB ▪ Measurement of intracellular ROS – DCFH assay	NHEK HaCaT RHEps	DCFH
LIPID PEROXIDATION	Assessment of anti-lipid peroxidation Inducer : UVB ▪ Measurement of MDA (malondialdehyde) level– TBAR Fluorescence assay	NHEK HaCaT	PEROX
FREE RADICALS INDUCED CYTOTOXICITY	Assessment of protective effects against free radical cytotoxicity Inducer : Hypoxanthine/xanthine oxidase (HX-XO) or UV ▪ Measurement of free radicals cytotoxicity - cell viability test (MTT)	NHDF HaCaT	RL-XO RL-UV
ANTIOXIDANT ENZYMES ACTIVITIES	Assessment of antioxidant potential – enzymes activities Measurement of antioxidant enzymes activities in cells after ROS-induced oxidative stress ▪ Superoxyde Dismutase activity (SOD) - Spectrophotometric assay. ▪ Catalase activity(CAT) - Spectrophotometric assay. ▪ Gluthation Peroxidase activity (GSH-PX) - Spectrophotometric assay. ▪ Gluthation Reductase activity(GSSG-R) - Spectrophotometric assay.	NHDF HaCaT	RLDEP
DPPH ANTI-RADICAL ACTIVITY	Assessment of Free Radical Scavenging - DPPH assay ▪ Measurement of reduction level of DPPH• - Spectrophotometric method	IN TUBO	DPPH
SUPEROXIDE ANTI-RADICAL ACTIVITY	Assessment of Free Radical Scavenging – ANTI-RADICAL SUPEROXIDE (O₂• -) ▪ Measurement of reduction level of nitro-blue tetrazolium (NBT) by O ₂ • in PMS/NADH system - Spectrophotometric method	IN TUBO	ARO2
HYDROXYL ANTI-RADICAL ACTIVITY	Assessment of Free Radical Scavenging - ANTI-RADICAL HYDROXYL (OH•) ▪ Measurement of oxidation level of dimethylsulfoxide (DMSO) by (•OH) - Spectrophotometric method	IN TUBO	AROH
ANTI-OXIDANT OXYGEN SINGLET ACTIVITY	Assessment of Free Radical Scavenging Effect – ANTI-SINGLET OXYGEN (¹O₂) ▪ Measurement of the bleaching of RNO by singlet oxygen (¹ O ₂) - Spectrophotometric method	IN TUBO	RNO

*cf. Appendix p. 11

STUDY	TEST PROCEDURE	CELL AND TISSUE MODELS*	STUDY CODE
ANTIOXIDANT / FREE RADICAL SCAVENGING (# part 2 : IN TUBO)			
ANTIRADICAL PEROXYL ACTIVITY	Assessment of antioxidant potential – ORAC Activity (oxygen radical absorbance capacity) ▪ Measurement of oxidation level of fluorescein (fluorescent probe) by peroxy radicals – Fluorimetric assay	IN TUBO	ORAC
LIPID PEROXIDATION	Assessment of anti-oxidant potential – Arachidonic Acid peroxidation (AAP) ▪ Measurement of the iron-mediated lipid peroxidation of arachidonic acid – Spectrophotometric assay	IN TUBO	AOX-TAA
ANTI-POLLUTION EFFICACY			
ANTI-OZONE PROTECTIVE EFFECT	Assessment of cytoprotective effect against ozone-induced toxicity Inducer : O ₃ ▪ Measurement of the production of TNF-α and IL-8 – Elisa assay	HaCat RHEPs	OZON OZON-ER
ANTI-CIGARETTE SMOKE PROTECTIVE EFFECT	Assessment of cytoprotective effect against cigarette smoke-induced cellular damages Inducer : CSE (cigarette smoke extract) ▪ Quantification of cell membrane integrity – NRU assay – Spectrophotometric assay	HaCat	CYFC
ANTI-HEAVY METALS PROTECTIVE EFFECT	Assessment of cytoprotective effect against metal-induced toxicity Inducer : Nickel (Ni), Lead (Pb) and Iron (Fe) ▪ Measurement of TNF-α production and release – Elisa assay	HaCat	EPM
ANTI-DIESEL PARTICULATE MATTER PROTECTIVE EFFECT	Assessment of cytoprotective effect against diesel particulate matter-induced toxicity Inducer : PM extract (diesel Particulate matter extract) ▪ Measurement of mitochondrial metabolism – MTT assay ▪ Quantification of intracellular ROS (reactive oxygen species) level – DCFH assay	HaCat RHEPs	PMDIEL PMDIEL-ER



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ANTI-INFLAMMATORY POTENTIAL /SOOTHING EFFECT			
INFLAMMATORY MEDIATORS	Assessment of modulating effects on inflammatory mediators production Inducer : UV, PMA, LPS, cytokines... <ul style="list-style-type: none"> ▪ Measurement of pro-inflammatory cytokines : IL-1α, IL-8, IL-6, TNF- α,... ▪ Measurement of arachidonic acid mediators : PGE2, LTB4,... 	HaCat NHEK RHEPs RAW 264.7	AAI-UV AAI-PMA AAI-LPS
DERMO-PROTECTIVE POTENTIAL	Assessment of epidermal protection against irritative reaction on RHE Inducer : SDS <ul style="list-style-type: none"> ▪ Measurement of pro-inflammatory cytokines IL-1α release 	RHEPs	DPSDS
FACTORS OF TRANSCRIPTION	Assessment of modulating effect on signal transduction Inducer : PMA, UV <ul style="list-style-type: none"> ▪ Measurement of transcription factor activation (phosphorylation, translocation) ▪ Factors of transcription : NFκB (Nuclear factor κB), AP-1, STAT-1, ERK1/2 	HaCat NHEK RAW 264.7	NFKB
NITRIC OXIDE PRODUCTION	Assessment of modulating effects on NO production Inducer : UV, LPS <ul style="list-style-type: none"> ▪ NO production : measurement of nitrite and nitrate levels – spectrophotometric assay ▪ iNOS expression - Western Blot assay 	HaCat NHEK RAW 264.7	NO / INOS
LOX-COX LIPOXYGENASE (LOX) / CYCLOXYGENASE (COX) ACTIVITIES	Assessment of modulating effect on LOX/COX activities in cells <ul style="list-style-type: none"> ▪ COX activity : measurement of 6-keto PGF1-α level in the supernatant – Elisa Assay ▪ LOX activity: measurement of LTB4 level in the supernatant – Elisa Assay ▪ COX-2 expression: measurement of cox-2 protein level - Western Blot assay 	NHEK HUVEC RAW 264.7 HaCaT	COX
	Assessment of modulating effect on 5-LOX /COX-1 / COX-2 activities -in tubo assays	IN TUBO	COX-T/ LOX-T
ICAM-1 PRODUCTION	Assessment of modulating effect on ICAM-1 expression Inducer : interferon- γ (IFN- γ) <ul style="list-style-type: none"> ➔RHEPs model: measurement of s-ICAM (<i>Soluble Intercellular adhesion molecule-1</i>) in incubation medium – Elisa assay ➔ Cellular model : measurement of ICAM-1 expression by immunocytochemistry 	HaCat NHEK RHEPs	ICAS ICAM

STUDY	TEST PROCEDURE	CELL AND TISSUE MODELS*	STUDY CODE
ENERGY METABOLISM / MITOCHONDRIAL CELLULAR RESPIRATION / CELL OXYGENATION			
CELLULAR ATP / NUCLEOTIDES	Assessment of modulating effect on energetic metabolism of cutaneous cells <ul style="list-style-type: none"> Measurement of cytosolic ATP and ADP rate in cell - Bioluminescence ATP assay Measurement of the level of mitochondrial ATP synthesis - Bioluminescence VATP assay 	NHDF HaCat NHEK RHEPs	ATPb V-ATP
CELLULAR RESPIRATION	Assessment of modulating effects on cell respiratory rate Inducer : basal / digitonin (permeabilized) / DNP (uncoupler) <ul style="list-style-type: none"> <i>Basal Cell Respiration</i> : measurement of oxygen consumption rate (VO₂) in basal condition <i>Mitochondrial respiration</i> : measurement of oxygen consumption rate (VO₂) in permeabilized and uncoupled cells 	NHDF HaCat	RCS-RCA
OXYGEN TRANSPORT/DONER EFFECTS	Assessment of modulating effects on cellular respiration under limited O₂ conditions. Inducer : Normoxia /Hypoxia <ul style="list-style-type: none"> Measurement of Oxygen consumption rate (VO₂) and [NAD⁺] / [NADH] concentration in cells 	NHDF HaCat	RCNAD
ATP - MITOCHONDRIAL STRESS	Assessment of modulating effects on epidermal mitochondrial dysfunction Inducer : sodium nitroprusside (SNP) – oxidant stress <ul style="list-style-type: none"> Measurement of ATP production - Bioluminescence ATP assay Measurement of mitochondrial membrane potential ($\Delta\psi_m$) – Fluorescence JC-1 assay 	HaCat	AMD
HAIR GROWTH AND HAIR PROTECTION			
HAIR GROWTH ACTIVATION	Assessment of the modulating effects on FGF-7 production and Wnt/β-catenin pathway activation in a [hair follicle cells/keratinocytes] co-culture cells <ul style="list-style-type: none"> Measurement of FGF-7 levels in conditioned media – Elisa assay Wnt/β-catenin activity of HFDPC cells – Elisa assay 	HFDPC/NHEK co-culture	HAIR-ACT
HAIR PROTECTION	Assessment of the protective effects against lipid peroxide-induced oxidative stress Inducer : LOOH (linolein hydroperoxide) <ul style="list-style-type: none"> Measurement of intracellular ROS : DCFH assay 	HFDPC	HAIR-PRO



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ANTI-AGEING / SKIN AGEING			
SIRTUIN ACTIVITY	<p>Assessment of modulating effects on Sirtuin activity</p> <ul style="list-style-type: none"> Measurement of SIRT-1 protein - Western Blot Measurement of SDAC activity (Sirtuin Deacetylase) – Fluorimetric SIRT-1 assay 	NHDF	SIRT
DERMOPROTECTION	<p>Assessment of protective effects against Epidermal (ROS protection) and Dermal ageing (Extracellular matrix synthesis and degradation)</p> <p>Inducer : UVB (epidermal ageing) / UVA (dermal ageing)</p> <ul style="list-style-type: none"> Measurement of ROS level – Fluorimetric DCFH assay Measurement of Procollagen type I C-peptide – Elisa assay Measurement of MMP-1 release – Elisa assay 	HaCat NHDF	EAA
	<p>Assessment of modulating effects on functional alterations in « aged » fibroblasts</p> <p>Inducer : chronic mitochondrial stress – FCCP exposure</p> <ul style="list-style-type: none"> Cellular proliferation : measurement of cell densities - colorimetric NRU assay Intracellular oxidation : measurement of ROS level – Fluorimetric DCFH assay Procollagen I synthesis : measurement of Procollagen type I C-peptide – Elisa assay Cytosolic ATP : measurement of ATP production - Bioluminescence ATPb assay Measurement of mitochondrial membrane potential ($\Delta\psi_m$) – Fluorimetric JC1 assay Cell Senescence : Measurement of senescence-associated β-galactosidase activity – microscopic β-Gal assay 	NHDF	FCCP
PROTEASOME ACTIVITY	<p>Assessment of modulating effects on Proteasome activity</p> <ul style="list-style-type: none"> Measurement of proteasome activity – Fluorescence 20S proteasome assay 	RHEps	PTA-ER
	<p>Assessment of modulating effects on mitochondrial Lon protease activity</p> <p>Inducer : acute oxidative stress H_2O_2</p> <ul style="list-style-type: none"> Measurement of mitochondrial Lon activity – Fluorescence mtLon assay 	NHDF	LON
SKIN PIGMENTATION (part#1)			
TYROSINASE ACTIVITY	<p>Assessment of modulating effects on tyrosinase activity</p> <ul style="list-style-type: none"> Measurement of tyrosinase activity (dopachrome formation) – Spectrophotometry assay 	NHEM	TYR
	<p>Assessment of tyrosinase inhibition activity on isolated tyrosinase (Mushroom)</p> <ul style="list-style-type: none"> Measurement of tyrosinase activity (dopachrome formation) – Spectrophotometry assay 	IN TUBO	ITC



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SKIN PIGMENTATION (part#2)			
MELANIN SYNTHESIS	<p>Assessment of modulating effects on melanogenesis ➔ Cellular model : Inducer : basal or MSH ▪ Measurement of melanin content – spectrophotometric assay ➔RHEPs model : Inducer : basal ▪ Measurement of melanin content – spectrophotometric assay ▪ Measurement of melanin content and coloration of reconstructed tanned eperdermis – calculation of individual typology angle (ITA°) – Chromametric assay</p> <p>Assessment of modulating effects on UV-activated melanogenesis Inducer : UVB ▪ Measurement of melanin content – spectrophotometric assay</p>	NHEM /B16 RHEps	MEL MEL-EPI
MELANOSOME TRANSFER	<p>Assessment of modulating effects on melanosome transfer CFDA transfert : ▪ Measurement of the fluorescent dye (CFDA) in keratinocytes fraction – Fluorimetric CFDA assay Pmel 17 : ▪ Measurement of melosomal protein (Pmel17) in keratinocytes fraction – Elisa assay</p>	Co-culture NHEM / NHEK	MEL- TRF MEL-TRF- Pmel
MELANOCYTE PROTEASOME ACTIVITY	<p>Assessment of modulating effects on Proteasome activity ▪ Measurement of proteasome activity – Fluorescence 20S proteasome assay</p>	NHEM RHEps	PTA PTA-ER
ANTI-ACNE			
5 α -REDUCTASE ACTIVITY	<p>Assessment of 5α-reductase activity ▪ Measurement of 5α-dehydrotestosterone (5α -DHT) level and/or by measurement of testosterone metabolites.</p>	Hs68 RHEps	REDF
β -DEFENSIN PRODUCTION	<p>Assessment of modulating effects on β-Defensin production Inducer : basal, LTA (Lipoteichoic acid) ▪ Measurement of β-Defensin level in cell culture supernatants – Elisa assay</p>	NHEK	HBD2
TNF- α PRODUCTION	<p>Assessment of modulating effects on TNF-α production Inducer : basal, LTA (Lipoteichoic acid) ▪ Measurement of TNF- α level in cell culture supernatants - Elisa assay</p>	NHEK	TNF



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TENSING /FIRMING ACTIVITY / DERMAL STRENGTH AND COHESION			
FIBROBLASTS DYNAMIC PROPERTIES	Assessment of strengthening effects on dynamic properties of fibroblasts. Kinetic study of dermis equivalent contraction by direct effect (addition in collagen gel) or indirect effect (treatment of cells before lattice formation) • Measurement of the lattice contraction –Imaging analysis	Dermis equivalent (collagen lattice)	CLT
SLIMMING EFFICACY			
LIPOLYTIC ACTIVITY	Assessment of modulating effects on lipolytic activity in basal or activated adipocytes • Measurement of triglyceride hydrolysis : free fatty acids release (Enzymatic assay) and glycerol release in culture supernatant (Enzymatic colorimetric assay) • Measurement of intracellular cyclic AMP level (cAMP)– Elisa assay	3T3-L1 preadipocytes Human adipocytes (ex vivo)	AL
ADIPOGENESIS	Assessment of modulating effects on adipogenesis by the evaluation of functional markers of adipocyte differentiation • Measurement of G3PDH activity - Enzymatic colorimetric assay • Measurement of intracellular lipids content – “Oil Red O” assay- colorimetric assay	3T3-L1 preadipocytes	ADIP
ADIPOSE TISSUE REGULATION			
ADIPOCYTES METABOLISM	Assessment of anti-adiposity effects by measuring 11β-hydroxysteroid deshydrogenase type 1 (11β-HSD1) activity • Measurement of cortisol level – Elisa assay	3T3-L1 preadipocytes	HSD
	Assessment of modulating effect on Adenylate cyclase activity • Measurement of cyclic AMP level (cAMP)– Elisa ACA assay	3T3-L1 adipocytes	ACA
ADIPOCYTES ACTIVATION	Evaluation of modulating effects on adipocytes activation in response to local inflammation Inducer : LPS • Measurement of adipokines release : IL-6, TNF- α , MCP-1 • Measurement of ROS production : DCFH assay • Measurement of NF κ B activation : Elisa-based assay	Co-culture 3T3-L1 adipocytes - macrophages	ADIPOK

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* Cell and tissue models

IN-VITRO EFFICACY

3T3-L1	3T3-L1 preadipocytes
B16	Murin B16 melanoma cell line
HaCat	Human keratinocyte cell line
HCE	Human corneal epithelium
HFDPC	Hair follicule dermal papilla cells
Hs 68	Human skin fibroblast cell line
HUVEC	Human umbilical vein endothelial cells
MDCK	Madin-darby canine kidney cell line
NHDF	Normal human dermal fibroblasts
NHEK	Normal human epidermal keratinocytes
NHEM	Normal epidermal melanocytes
RAW 264.7	Mouse macrophages cell line
RHEs	Reconstructed human epidermis
RHEps	Reconstructed human epidermis pigmented
Skin equivalent	Epidermal compartment at the surface of a dermal equivalent (collagen lattis)