gibco

Skin and eye primary cell sourcebook

Experience consistent predictions from your primary cell culture

ThermoFisher SCIENTIFIC

To be the best, use the best

Gibco™ cell culture products are infused with quality, customer-focused innovation, and service excellence from beginning to end. From the most basic formulations to the latest innovations, Gibco products are designed to deliver superior quality, consistency, and performance—for results that you can count on every day.

Our cells are ethically sourced

We work with a variety of human tissue sources, including tissue and organ procurement organizations, qualified research tissue organizations, and prominent academic and medical centers through collaborations that follow rigorous regulations, certifications, and/or accreditations. Tissues obtained through these source facilities are consistent with the legal and ethical practices of the United States and European Union. As such, we follow these regulations and meet or exceed these standards. Specifically, we assure that all consents for the use of human cells derived from these tissues have been obtained from the next of kin.

Contents

Cutaneous cell culture

Introduction to cutaneous cell culture

Human skin is the largest organ of the body, accounting for ~15% of body weight. Together with various other components such as glands, fingernails, and hair, it comprises a complex system known as the integumentary system. Human skin performs a number of diverse functions critical to normal health, including thermoregulation, protection of internal organs, and providing a physical barrier against environmental insults such as pathogens and radiation from the sun. In addition, skin helps prevent dehydration, possesses metabolic activity (vitamin D production), delivers touch, heat, and pain sensations via the peripheral nervous system, excretes salts and wastes, and aids in wound healing.

Research applications for cutaneous cell systems are shown in Table 1.

Primary skin cell systems

Skin is composed of two layers: the dermis and epidermis, each with unique components and functions (Figure 1). The epidermis or outermost layer of the skin consists primarily of epithelial cells, specifically keratinocytes, which form a stratified layer and produce keratins to harden and waterproof the skin. The epidermis contains other cell types, including melanocytes and Langerhans cells. Melanocytes comprise ~5% of the cells in the basal layer of the epidermis and function primarily to produce melanin, which provides pigmentation for both hair and skin and protection from UV radiation.

Melanocytes intercalate with the epidermis and establish close and critical interactions with keratinocytes to perform various cellular functions during development and normal maintenance of the skin.

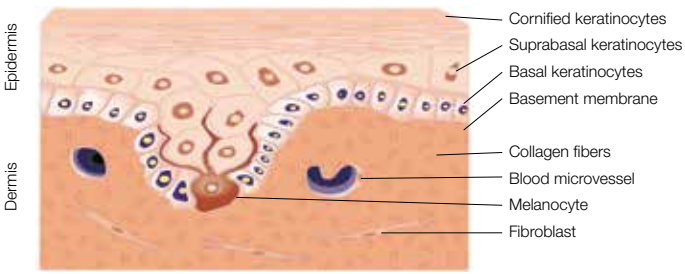


Figure 1. Components of skin.

Table 1. Research applications for cutaneous cell systems.

Applications
Basic structure/function studies
Dermal modeling
Gene regulation
Signal transduction
Skin cell co-culturing
Cancer biology
Angiogenesis
Melanoma
Normal controls
Drug discovery/cosmetics/beauty and personal care studies
Acne
HTS/HCA screening
Pigmentation
Secondary and tertiary screens
Toxicology screening
***In vitro* alternatives to animal testing**
Corrosivity
Cosmetics and topicals
Household products
Irritancy
Safety assessment testing services and products
Cell therapy
Burn therapy
Chronic skin ulcers
Cosmetic (wrinkles, scars, hair growth)
Wound healing

Applications for primary skin cell systems

The dermis, unlike the epidermis, is vascularized and provides nutrients to the outermost layer of the skin via diffusion. The dermal compartment also provides structural support for the skin mediated by an extracellular matrix (ECM), which is principally composed of collagen and elastin fibers. Fibroblasts are the main cell type in dermis and are responsible for production of ECM proteins, which impart the skin with much of its mechanical and elastic strength. Layered within the ECM are dermal microvasculature and lymphatic vessels for blood circulation and waste removal, vital to proper skin function.

The four major types of cutaneous cells are shown in Figure 2.

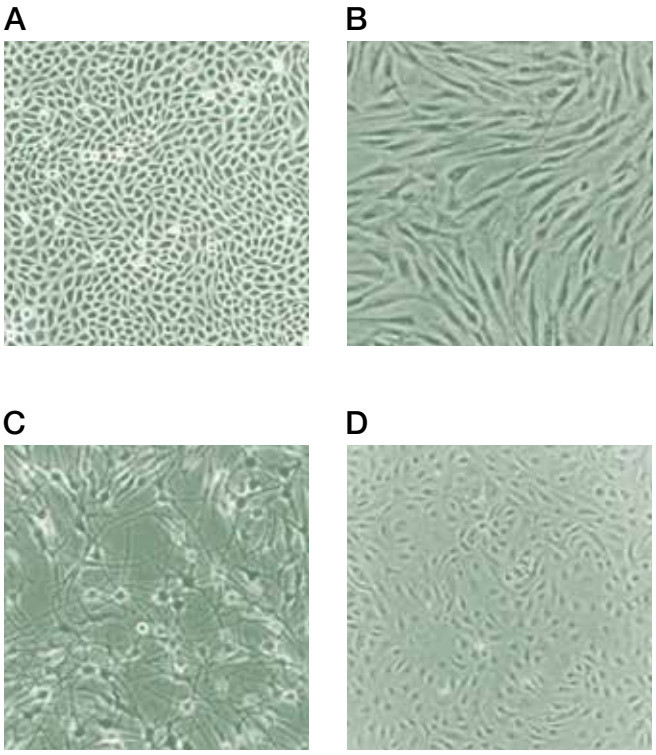


Figure 2. Types of cutaneous cells. (A) Human epidermal keratinocytes (HEK). **(B)** Human dermal fibroblasts (HDF). **(C)** Human epidermal melanocytes (HEM). **(D)** Human dermal microvascular endothelial cells (HMVEC).

Key dermal cell culture products

When you demand robust and relevant tools for your primary cell culture work, select from these and other key dermal cell culture products (Table 2), or visit **thermofisher.com/primarycells** for more details.

Table 2. Products for dermatological research.*

Primary human cells (cryopreserved)	Keratinocytes • Neonatal (C-001-5C) • Adult (C-005-5C) • Pooled (A13401)	Melanocytes • Neonatal, lightly pigmented donor (C-002-5C) • Neonatal, moderately pigmented donor (C-102-5C) • Neonatal, darkly pigmented donor (C-202-5C) • Adult, lightly pigmented donor (C-024-5C)	Dermal fibroblasts • Neonatal (C-004-5C) • Adult (C-013-5C)	Dermal microvascular endothelial cells • Neonatal (C-010-5C) • Adult (C-011-5C)
	Keratinocytes (prepared APF) • Neonatal (C-020-5C) • Adult (C-021-5C)			
Basal media	EpiLife™ medium (500 mL) • Standard (M-EPI-500-CA) • Calcium-free (M-EPIcf-500) • Calcium- and phenol red–free (M-EPIcf/PRF-500)	Medium 254 (500 mL) • Standard (M-254-500) • Calcium-free (M-254CF-500)	Medium 106 (500 mL) (M-106-500)	Medium 131 with attachment factor (500 mL) (M-131-500)
	Medium 154 (500 mL) • Standard (M154-500) • Calcium-free (M154-CF-500) • Calcium- and phenol red–free (M154-CF/PRF-500)			
Growth supplements	Human keratinocyte growth supplement (HKGS) • Single-addition (S-001-5) • Kit (S-001-K)	Human melanocyte growth supplement (HMGS) (S-002-5)	Low-serum growth supplement (LSGS) • Single-addition (S-003-10) • Kit (S-003-K)	Microvascular growth supplement (MVGS) (S-005-25)
	EpiLife defined growth supplement (EDGS) (S-012-5)†	Human melanocyte growth supplement-2 (HMGS-2) (S-016-5)		Attachment factor (100 mL) (S-006-100)
	Supplement S7† (S-017-5)			
	Subculture and other reagents			
	Coating Matrix Kit (R-011-K) Defined Trypsin Inhibitor (R-007-100) Gentamicin/Amphotericin Solution (R-015-10) TrypLE™ Express Enzyme (12604-013) Synth-a-Freeze™ Cryopreservation Medium (A1254201) Geltrex™ LDEV-Free Reduced Growth Factor Basement Membrane Matrix (A1413201)			

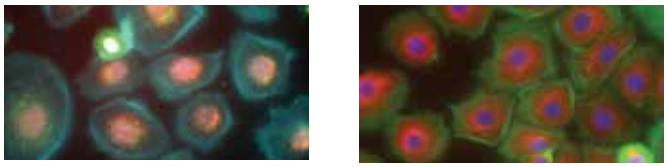
* The cells listed in Table 2 are also available, in the United States only, as proliferating cultures (catalog numbers for proliferating cultures take the form C-xxx-25P). All cells have tested negative for HIV-1, hepatitis B, hepatitis C, mycoplasmas, bacteria, yeast, and other fungi and are highly characterized.
† Requires use of Coating Matrix Kit.

Keratinocyte cell culture overview

We offer a wide array of Gibco™ products for keratinocyte culture, including products that are free of any animal-derived components such as bovine pituitary extract (BPE), serum, or other components that are typically purified from animal sources. We refer to these products as being “animal product–free” and use the abbreviation “AFP” to identify them. We offer complete cell culture systems designed and optimized to work together for the study of keratinocytes. For a complete review of Gibco™ primary cell technologies and services, visit **thermofisher.com/primarycells**.

Keratinocyte specifications

Gibco™ neonatal cells are able to grow through at least 30 population doublings when cultured in Gibco™ EpiLife™ Medium supplemented with HKGS (for HEKn) or Supplement S7 (for HEKn-APF). Adult cells are able to grow through at least 25 population doublings when cultured in EpiLife Medium supplemented with HKGS (for HEKa) or Supplement S7 (for HEKa-APF).



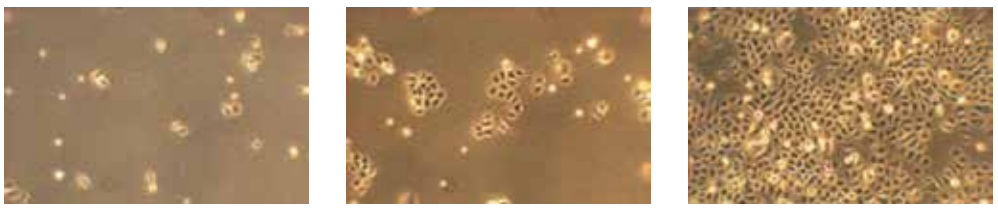
Fluorescent multiplex imaging of neonatal HEKs (false colored). **(A)** Anti–PMP 70 peroxisomal marker antibody/Alexa Fluor™ 647 goat anti–rabbit secondary antibody (orange); anti–golgin 97 antibody/Alexa Fluor™ 555 goat anti–rabbit secondary antibody (green); Alexa Fluor™ 488 phalloidin (cyan); Hoechst 33342 (pink). **(B)** Anti–α-tubulin antibody/Alexa Fluor 555 goat anti–rabbit secondary antibody (red); Alexa Fluor 488 phalloidin (green); Hoechst 33342 (blue).

Research applications
Basic dermal biology/physiology
Cosmetics/consumer products testing
Dermal research and models
Drug/compound screening
Drug discovery projects
Hair growth and replacement
High-throughput screening
Melanoma research
Pathogen and barrier function studies
Toxicity testing

Recommended culture systems

Culture environment			
	Animal origin–free	**Chemically defined**	**BPE-containing**
Cells	HEKn-AOF or HEKa-AOF	HEKn, HEKa, or HEKp	HEKn, HEKa, or HEKp
Basal medium	EpiLife	EpiLife	EpiLife
Growth supplement	Supplement S7	EDGS	HKGS
Reagents	TrypLE Express, Defined Trypsin Inhibitor, gentamicin/amphotericin, Synth-a-Freeze, Coating Matrix Kit	Trypsin/EDTA, Defined Trypsin Inhibitor, gentamicin/amphotericin, Synth-a-Freeze, Coating Matrix Kit	Trypsin/EDTA, Trypsin Neutralizer Solution, gentamicin/amphotericin, Synth-a-Freeze

Cultured in EpiLife Medium + HKGS



Cultured in Medium 154 + HKGS

HEKn, day 1 **HEKn, day 3** **HEKn, day 5**

Comparison of HEKn (Cat. No. C-001-5C), secondary culture, grown in either EpiLife Medium or Medium 154.

Keratinocyte cells

Human Epidermal Keratinocytes (HEK), neonatal cells

HEKn, cryopreserved

Normal human epidermal keratinocytes isolated from neonatal foreskin, cryopreserved at the end of the primary culture.

Ordering information

Quantity	Cat. No.
1 vial (>500,000 viable cells)	C-001-5C

HEKn-APF, cryopreserved

Normal human epidermal keratinocytes from neonatal foreskin isolated, grown, and cryopreserved in an animal product–free environment. Cryopreserved at the end of the primary culture. For optimal performance when culturing keratinocytes in an animal product–free environment, we recommend coating the culture surfaces with our Gibco™ Coating Matrix Kit (Cat. No. R-011-K).

Ordering information

Quantity	Cat. No.
1 vial (>500,000 viable cells)	C-020-5C

HEKp, cryopreserved

Normal human epidermal keratinocytes isolated from multiple neonatal foreskins and cryopreserved at the end of the primary culture stage in AOF medium containing 10% DMSO.

Ordering information

Quantity	Cat. No.
1 vial (1 x 10^6 viable cells)	A13401

HEKn, proliferating*

Secondary cultures established from cryopreserved cells, grown to approximately 50% confluence in T-flasks using the following medium and supplement combinations:

Ordering information

Product	Quantity	Cat. No.
HEKn, proliferating culture, prepared in EpiLife Medium and HKGS	6 x T-25 flasks	C-001-25P-A
HEKn, proliferating culture, prepared in EpiLife Medium and EDGS	6 x T-25 flasks	C-001-25P-B
HEKn, proliferating culture, prepared in EpiLife Medium and Supplement S7	6 x T-25 flasks	C-001-25P-C
HEKn, proliferating culture, prepared in EpiLife-PRF Medium and HKGS	6 x T-25 flasks	C-001-25P-D
HEKn, proliferating culture, prepared in EpiLife-PRF Medium and EDGS	6 x T-25 flasks	C-001-25P-E
HEKn, proliferating culture, prepared in EpiLife-PRF Medium and Supplement S7	6 x T-25 flasks	C-001-25P-F
HEKn, proliferating culture, prepared in Medium 154 and HKGS	6 x T-25 flasks	C-001-25P-G
HEKn, proliferating culture, prepared in Medium 154PRF and HKGS	6 x T-25 flasks	C-001-25P-H

*Setup required. Proliferating cultures are currently available in the US only.

Human Epidermal Keratinocytes (HEK), adult cells

HEKa, cryopreserved

Normal human epidermal keratinocytes, isolated from adult skin, cryopreserved at the end of the primary culture.

Ordering information

Quantity	Cat. No.
1 vial (>500,000 viable cells)	C-005-5C

HEKa-APF, cryopreserved

Normal human epidermal keratinocytes from adult skin. Isolated, grown, and cryopreserved in an animal product–free environment. Cryopreserved at the end of the primary culture. For optimal performance when culturing keratinocytes in an animal product–free environment, we recommend coating the culture surfaces with our Coating Matrix Kit (Cat. No. R-011-K).

Ordering information

Quantity	Cat. No.
1 vial (>500,000 viable cells)	C-021-5C

HEKa, proliferating*

Secondary cultures established from cryopreserved cells, grown to approximately 50% confluence in T-flasks using the following medium and supplement combinations:

Ordering information

Product	Quantity	Cat. No.
HEKa, proliferating culture, prepared in EpiLife Medium and HKGS	6 x T-25 flasks	C-005-25P-A
HEKa, proliferating culture, prepared in EpiLife Medium and EDGS	6 x T-25 flasks	C-005-25P-B
HEKa, proliferating culture, prepared in EpiLife Medium and Supplement S7	6 x T-25 flasks	C-005-25P-C
HEKa, proliferating culture, prepared in EpiLife-PRF Medium and HKGS	6 x T-25 flasks	C-005-25P-D
HEKa, proliferating culture, prepared in EpiLife-PRF Medium and EDGS	6 x T-25 flasks	C-005-25P-E
HEKa, proliferating culture, prepared in EpiLife-PRF Medium and Supplement S7	6 x T-25 flasks	C-005-25P-F
HEKa, proliferating culture, prepared in Medium 154 and HKGS	6 x T-25 flasks	C-005-25P-G
HEKa, proliferating culture, prepared in Medium 154PRF and HKGS	6 x T-25 flasks	C-005-25P-H

*Setup required. Proliferating cultures are currently available in the US only.

Keratinocyte media

Basal media for keratinocytes

EpiLife Medium is designed for extended lifespan of keratinocytes. If the desired endpoint is differentiation, Gibco™ Medium 154 may provide better results. Media do not contain antibiotics or antimycotics.

EpiLife Medium

Get the most from your cells using EpiLife serum-free, chemically defined, animal origin–free cell culture medium. EpiLife Medium can extend the *in vitro* lifespan of primary cells in culture up to twice as long compared to other serum-free formulations (Figure 3). EpiLife Medium contains 60 µM $CaCl_2$ and is convenient and easy to use with single-shot supplementation. It is ideal for supporting the isolation, growth, and survival of both normal human keratinocytes and other types of epithelial cells when combined with appropriate supplements.

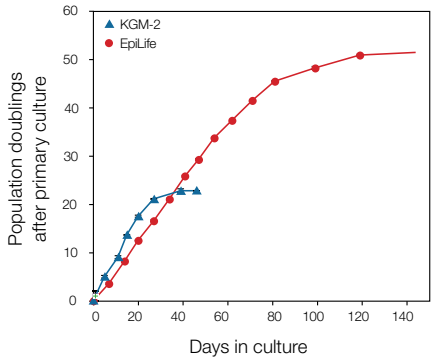


Figure 3. Normal neonatal human keratinocytes (Cat. No. C0015C) were grown in EpiLife Medium and in a keratinocyte medium from a leading competitor. Cultures grown in EpiLife Medium demonstrated population doublings over an extended period compared to cells grown in the competitor's medium.

Ordering information

Quantity	Cat. No.
500 mL	M-EPI-500-CA

EpiLife CF (calcium-free)

A sterile, liquid medium prepared without calcium chloride* for the long-term, serum-free culture of human epidermal keratinocytes. This basal medium requires the addition of calcium plus an appropriate growth supplement prior to use. Calcium chloride is provided as a separate component with each bottle of medium.

Ordering information

Quantity	Cat. No.
500 mL	M-EPI-500

EpiLife CF/PRF (calcium-free, phenol red–free)

A sterile, liquid medium for the long-term, serum-free culture of human epidermal keratinocytes. Gibco™ EpiLife CF/PRF is EpiLife Medium prepared without calcium chloride* and phenol red. This basal medium requires the addition of calcium plus an appropriate growth supplement prior to use. Calcium chloride is provided as a separate component with each bottle of medium.

Ordering information

Quantity	Cat. No.
500 mL	M-EPICF/PRF-500

Medium 154

A sterile, liquid medium for the serum-free culture of human epidermal keratinocytes. This basal medium requires the addition of HKGS (Cat. No. S-001-5) or HKGS Kit (Cat. No. S-001-K) prior to use. Contains 200 µM calcium chloride.

Ordering information

Quantity	Cat. No.
500 mL	M-154-500

Medium 154CF (calcium-free)

A sterile, liquid medium for the serum-free culture of human epidermal keratinocytes. Gibco™ Medium 154CF is Medium 154 prepared without calcium chloride.† This basal medium requires the addition of calcium plus HKGS (Cat. No. S-001-5) or HKGS Kit (Cat. No. S-001-K) prior to use. Calcium chloride is provided as a separate component with each bottle of medium.

Ordering information

Quantity	Cat. No.
500 mL	M-154CF-500

Medium 154CF/PRF (calcium-free, phenol red-free)

A sterile, liquid medium for the serum-free culture of human epidermal keratinocytes. Gibco™ Medium 154CF/PRF is Medium 154 prepared without calcium chloride† and phenol red. This basal medium requires the addition of calcium plus HKGS (Cat. No. S-001-5) or HKGS Kit (Cat. No. S-001-K) prior to use. Calcium chloride is provided as a separate component with each bottle of medium.

Ordering information

Quantity	Cat. No.
500 mL	M-154CF/PRF-500

* Calcium concentration from other sources is 0.65 µM in unsupplemented EpiLife CF and CF/PRF.

† Calcium concentration from other sources is 0.5 µM in unsupplemented Medium 154CF and Medium 154CF/PRF.

Keratinocyte supplements and reagents

Growth supplements for keratinocytes

Only supplements in kit form contain antibiotics and antimycotics.

Human Keratinocyte Growth Supplement (HKGS)

A sterile, concentrated (100X) solution intended for use with EpiLife Medium or Medium 154 to culture human epidermal keratinocytes. Contains bovine pituitary extract (BPE),* human epidermal growth factor, hydrocortisone, recombinant human insulin-like growth factor-1 (IGF-1), and transferrin.

Ordering information

Quantity	Cat. No.
5 mL	S-001-5

*BPE from New Zealand and/or Australian sources only.

Human Keratinocyte Growth Supplement (HKGS) Kit

A sterile set of solutions intended for use with EpiLife Medium or Medium 154 to culture human epidermal keratinocytes. The HKGS Kit provides, in separate vials, all the components of complete HKGS: bovine pituitary extract (BPE),* human epidermal growth factor, hydrocortisone, recombinant human insulin-like growth factor-1 (IGF-1) and transferrin. A vial of gentamicin/amphotericin B solution (GA) is also included. Use of GA is optional.

Ordering information

Quantity	Cat. No.
1 kit	S-001-K

*For use with EpiLife Medium or Medium 154.

EpiLife Defined Growth Supplement (EDGS)

A defined, sterile, concentrated (100X) solution intended for use with EpiLife Medium to culture human epidermal keratinocytes (not intended for use with Medium 154).

Contains BSA, bovine transferrin, rhIGF-1, rhEGF, hydrocortisone, and PGE-2 (synthetic).

For optimal performance we recommend using EDGS in conjunction with our Coating Matrix Kit (Cat. No. R-011-K).

Ordering information

Quantity	Cat. No.
1 kit	S-012-5

Recommended reagents		
Human Keratinocyte Growth Supplement (HKGS)	5 mL, 100X	S-001-5
HKGS Kit, includes components of HKGS separately, including a vial of GA	1 kit	S-001-K
Trypsin/EDTA Solution	100 mL	R-001-100
EpiLife Defined Growth Supplement (EDGS)	5 mL, 100X	S-012-5
Supplement S7	5 mL	S-017-5
Trypsin Neutralizer Solution	100 mL	R-002-100
Coating Matrix Kit	1 kit	R-011-K
Gentamicin/Amphotericin Solution	10 x 1 mL	R-015-10
Keratinocyte AOF Growth Kit	1 kit	A1051501

Supplement S7

A defined, sterile, concentrated (100X), ionically balanced solution intended for use with EpiLife Medium to culture human epidermal keratinocytes (not intended for use with Medium 154). For optimal performance we recommend using Supplement S7 in conjunction with our Coating Matrix Kit (Cat. No. R-011-K).

Ordering information

Quantity	Cat. No.
5 mL	S-017-5

Keratinocyte AOF Growth Kit

For the animal origin–free culture of human keratinocytes. Contains 1 each of EpiLife Basal Medium, Supplement S7, and Coating Matrix Kit.

Ordering information

Quantity	Cat. No.
1 kit	A1051501

Melanocyte cell culture overview

We offer a variety of Gibco™ melanocyte primary cells, with light, moderate, and dark pigmentations, in addition to complete cell culture systems, which are designed and optimized to work together. Both proliferating and cryopreserved cells are available (proliferating cultures only available in the US). For a complete review of Gibco primary cell technologies and services, visit **thermofisher.com/primarycells**.

Melanocyte specifications

Gibco™ HEMn-MP and HEMn-DP are able to grow through at least 12 population doublings; HEMn-LP and HEMa-LP are able to grow through at least 16 population doublings. HEMa-LP stain positively with Mel-5 antibody in the fourth culture after thawing.

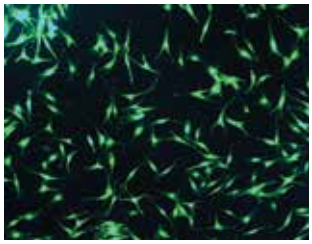
Recommended culture systems	
Cells	HEMa-LP
Basal medium	Medium 254
Growth supplements	HMGS, HMGS-2
Reagents	Trypsin/EDTA Trypsin Neutralizer Solution Gentamicin/amphotericin

Research applications
Basic dermal biology/physiology
Dermal research and models
High-throughput screening
Melanoma research
Pigmentation and related disorders

Melanocyte cells

Characterization of human epidermal melanocytes

Each lot of cells is performance tested in our laboratory for viability and growth potential. The cells are also tested for potential biological contaminants (HIV-1, hepatitis B, and hepatitis C viruses, mycoplasmas, bacteria, yeast, and other fungi). To be approved for distribution, cells must be at least 70% viable upon thawing, each vial must contain at least 500,000 viable cells, and no potential biological contaminants must be detected.



HEMa, day 9 Mel-5 immunofluorescence.

HEMa, day 9 phase contrast.

Human Epidermal Melanocytes (HEM), neonatal cells

HEMn-LP, cryopreserved normal human epidermal melanocytes isolated from lightly pigmented neonatal foreskin, cryopreserved at the end of the secondary culture.

Ordering information

Quantity	Cat. No.
1 vial (>500,000 viable cells)	C-002-5C

HEMn-LP, proliferating*

Tertiary cultures established from cryopreserved HEMn-LP, grown to approximately 50% confluence in T-flasks using the following medium and supplement combinations:

Ordering information

Product	Quantity	Cat. No.
HEMn-LP, proliferating culture, prepared in Medium 254 and HMGS	3 x T-25 flasks	C-002-25P-A
HEMn-LP, proliferating culture, prepared in Medium 254 and HMGS-2	3 x T-25 flasks	C-002-25P-B

*Setup required. Proliferating cultures are currently available in the US only.

HEMn-MP, cryopreserved

Normal human epidermal melanocytes isolated from moderately pigmented neonatal foreskin, cryopreserved at the end of the secondary culture.

Ordering information

Quantity	Cat. No.
1 vial (>500,000 viable cells)	C-102-5C

HEMn-MP, proliferating*

Tertiary cultures established from cryopreserved HEMn-MP, grown to approximately 50% confluence in T-flasks using the following medium and supplement combinations:

Ordering information

Product	Quantity	Cat. No.
HEMn-LP, proliferating culture, prepared in Medium 254 and HMGS	3 x T-25 flasks	C-102-25P-A
HEMn-LP, proliferating culture, prepared in Medium 254 and HMGS-2	3 x T-25 flasks	C-102-25P-B

*Setup required. Proliferating cultures are currently available in the US only.

Human Epidermal Melanocytes (HEM), adult cells

HEMa-LP, cryopreserved

Normal human epidermal melanocytes isolated from lightly pigmented adult skin, cryopreserved at the end of the secondary culture. For optimal performance when culturing adult melanocytes, we recommend using Gibco™ Human Melanocyte Growth Supplement-2 (Cat. No. S-016-5).

Ordering information

Quantity	Cat. No.
1 vial (>500,000 viable cells)	C-024-5C

HEMn-DP, cryopreserved

Normal human epidermal melanocytes isolated from darkly pigmented neonatal foreskin, cryopreserved at the end of the secondary culture.

Ordering information

Quantity	Cat. No.
1 vial (>500,000 viable cells)	C-202-5C

HEMn-DP, proliferating*

Tertiary cultures established from cryopreserved HEMn-DP, grown to approximately 50% confluence in T-flasks using the following medium and supplement combinations:

Ordering information

Product	Quantity	Cat. No.
HEMn-LP, proliferating culture, prepared in Medium 254 and HMGS	3 x T-25 flasks	C-202-25P-A
HEMn-LP, proliferating culture, prepared in Medium 254 and HMGS-2	3 x T-25 flasks	C-202-25P-B

*Setup required. Proliferating cultures are currently available in the US only.

HEMa-LP, proliferating*

Tertiary cultures established from cryopreserved HEMa-LP, grown to approximately 50% confluence in T-flasks using the following medium and supplement combinations:

Ordering information

Product	Quantity	Cat. No.
HEMa-LP, proliferating culture, prepared in Medium 254 and HMGS	3 x T-25 flasks	C-024-25P-A
HEMa-LP, proliferating culture, prepared in Medium 254 and HMGS-2	3 x T-25 flasks	C-024-25P-B

*Setup required. Proliferating cultures are currently available in the US only.

Melanocyte media

Basal media for melanocytes

Media do not contain antibiotics or antimycotics.

Medium 254

A sterile liquid medium for the culture of human epidermal melanocytes. This basal medium requires the addition of HMGS (Cat. No. S-002-5) or HMGS-2 (Cat. No. S-016-5) prior to use.

Ordering information

Quantity	Cat. No.
500 mL	M-254-500

Medium 254CF (calcium-free)

A sterile, liquid medium for the culture of human epidermal melanocytes. Gibco™ Medium 254CF is Medium 254 prepared without calcium chloride.* Calcium chloride is provided as a separate component with each bottle of medium. This basal medium requires the addition of calcium plus HMGS (Cat. No. S-002-5) or HMGS-2 (Cat. No. S-016-5) prior to use.

Ordering information

Quantity	Cat. No.
500 mL	M-254CF-500

* Calcium concentration from other sources is 0.5 µM in unsupplemented Medium 254CF.

Melanocyte supplements and reagents

Growth supplements for melanocytes

Supplements do not contain antibiotics or antimycotics.

Human Melanocyte Growth Supplement (HMGS)

A sterile, concentrated (100X) solution intended for use with Medium 254 or Medium 254CF to culture human epidermal melanocytes. Contains: fetal bovine serum, basic fibroblast growth factor, bovine pituitary extract (BPE),* heparin, hydrocortisone, recombinant human insulin-like growth factor-1 (IGF-1), transferrin, and phorbol 12-myristate 13-acetate. Recommended for either neonatal or adult melanocytes.

Ordering information

Quantity	Cat. No.
5 mL	S-002-5

Human Melanocyte Growth Supplement-2 (HMGS-2)

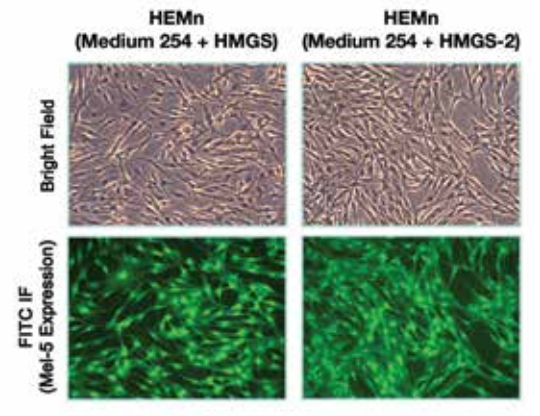
A sterile, concentrated (100X) solution intended for use with Medium 254 or Medium 254CF to culture human epidermal melanocytes.

Contains fetal bovine serum, basic fibroblast growth factor, bovine pituitary extract (BPE),* heparin, hydrocortisone, recombinant human insulin-like growth factor-1 (IGF-1), transferrin, and endothelin-1. Recommended for HEMa-LP.

Ordering information

Quantity	Cat. No.
5 mL	S-016-5

* BPE from New Zealand and/or Australian sources only.



Recommended reagents		
Gentamicin/Amphotericin Solution	10 x 1 mL	R-015-10
TrypLE Express Enzyme	100 mL	12604-013
Trypsin/EDTA Solution	100 mL	R-001-100
Trypsin Neutralizer Solution	100 mL	R-002-100

Human dermal fibroblast cell culture overview

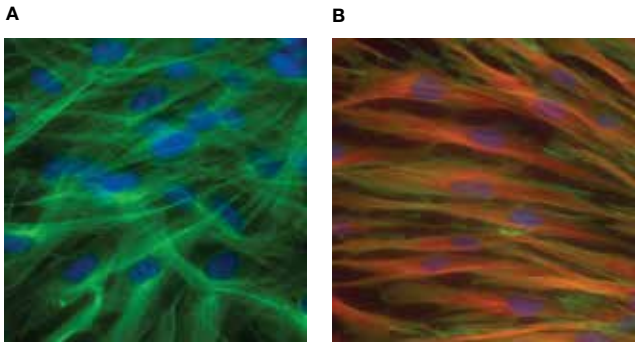
We offer both Gibco™ adult and neonatal fibroblast primary cells. Gibco™ complete cell culture systems are optimized and designed to work together for the study of dermal fibroblasts. Both proliferating and cryopreserved cells are available (proliferating cultures only available in the US). For a complete review of Gibco primary cell technologies and services, visit **thermofisher.com/primarycells**.

Research applications
Basic dermal biology/physiology
Co-culturing with dermal cell types
Dermal research and models
ECM and basement membrane research
High-throughput screening
Induced pluripotent stem cell studies
Wound healing

Fibroblast specifications

Gibco™ HDFa are able to grow through at least 12 population doublings, and Gibco™ HDFn are able to grow through at least 16 population doublings.

Recommended culture systems	
Cells	HDFa
Basal medium	Medium 106
Growth supplements	LSGS
Reagents	Trypsin/EDTA Trypsin Neutralizer Solution Gentamicin/amphotericin



Fluorescent multiplex imaging of microfilaments in human dermal fibroblasts. (A) Alexa Fluor 488 phalloidin (green); Hoechst 33342 (blue). **(B)** Anti–α-tubulin antibody/Alexa Fluor 555 goat anti-rabbit secondary antibody (red); Alexa Fluor 488 phalloidin (green); Hoechst 33342 (blue).

Human dermal fibroblast cells

Characterization of human dermal fibroblasts

Each lot of cells is performance tested in our laboratory for viability and growth potential. The cells are also tested for potential biological contaminants (HIV-1, hepatitis B, and hepatitis C viruses, mycoplasmas, bacteria, yeast, and other fungi). To be approved for distribution, cells must be at least 70% viable upon thawing, each vial must contain at least 500,000 viable cells, HDFa must be able to grow through at least 12 population doublings, HDFn must be able to grow through at least 16 population doublings, and no potential biological contaminants must be detected. Certificates of Analysis are available on our website, or by request.

Human Dermal Fibroblasts (HDF), neonatal cells

HDFn, cryopreserved

Normal human dermal fibroblasts isolated from neonatal foreskin, cryopreserved at the end of the primary culture.

Ordering information

Quantity	Cat. No.
1 vial (>500,000 viable cells)	C-004-5C

HDFn, proliferating*

Secondary cultures established from cryopreserved cells, grown to approximately 50% confluence in T-flasks using the following medium and supplement combination:

Ordering information

Product	Quantity	Cat. No.
HDFn, proliferating culture, prepared in Medium 106 and LSGS	6 x T-25 flasks	C-004-25P-A

*Setup required. Proliferating cultures are currently available in the US only.

Human Dermal Fibroblasts (HDF), adult cells

HDFa, cryopreserved

Normal human dermal fibroblasts isolated from adult skin, cryopreserved at the end of the primary culture.

Ordering information

Quantity	Cat. No.
1 vial (>500,000 viable cells)	C-013-5C

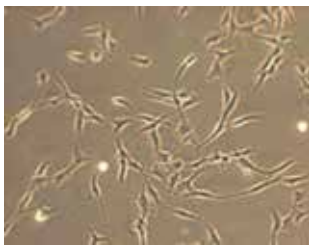
HDFa, proliferating*

Secondary cultures established from cryopreserved cells, grown to approximately 50% confluence in T-flasks using the following medium and supplement combination:

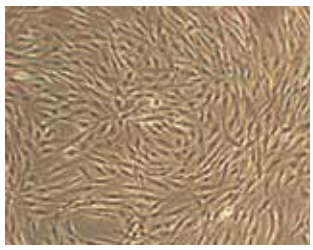
Ordering information

Product	Quantity	Cat. No.
HDFa, proliferating culture, prepared in Medium 106 and LSGS	6 x T-25 flasks	C-013-25P-A

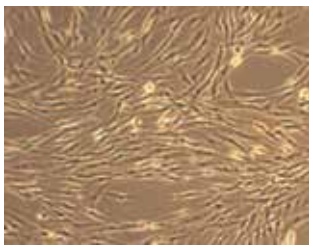
*Setup required. Proliferating cultures are currently available in the US only.



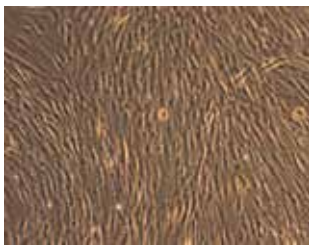
HDFn, day 3



HDFn, day 5



HDFa, day 5



HDFa, day 8

Human dermal fibroblast media

Basal medium for fibroblasts

Medium does not contain antibiotics or antimycotics.

Medium 106

A sterile, liquid medium for the culture of human dermal fibroblasts. This basal medium requires the addition of Gibco™ LSGS (Cat. No. S-003-10) or LSGS Kit (Cat. No. S-003-K) prior to use.

Ordering information

Quantity	Cat. No.
500 mL	M-106-500

Human dermal fibroblast supplements and reagents

Growth supplements for fibroblasts

Only supplements in kit form contain antibiotics/ antimycotics.

Low Serum Growth Supplement (LSGS)

A sterile, concentrated (50X) solution intended for use with Medium 106 (fibroblasts) or Medium 200 (endothelial cells). Optimized for dermal fibroblast culture when paired with Medium 106, LSGS contains fetal bovine serum, basic fibroblast growth factor, heparin, hydrocortisone, and epidermal growth factor.

Ordering information

Quantity	Cat. No.
10 mL	S-003-10

Low Serum Growth Supplement (LSGS) Kit

The LSGS Kit provides, in separate vials, all the components of complete LSGS: fetal bovine serum, hydrocortisone, human epidermal growth factor, and basic fibroblast growth factor/heparin (stabilized with BSA). A vial of gentamicin/amphotericin B solution (GA) is also included. Use of GA is optional.

Ordering information

Quantity	Cat. No.
1 kit	S-003-K

Recommended reagents		
TrypLE Express Enzyme	100 mL	12604-013
Trypsin Neutralizer Solution	100 mL	R-002-100
Trypsin/EDTA Solution	100 mL	R-001-100
Coating Matrix Kit	1 kit	R-011-K

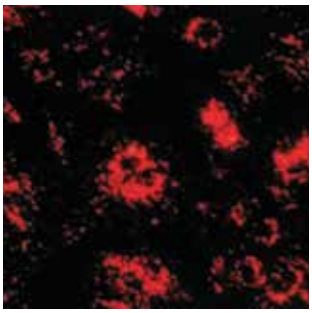
Microvascular endothelial cell culture overview

We offer both adult and neonatal Gibco™ human microvascular endothelial cells (HMVECs). Our complete Gibco™ cell culture systems are optimized and designed to work together for the study of HMVECs. Both proliferating and cryopreserved cells are available (proliferating cultures only available in the US). For a complete review of primary cell technologies and services, visit **thermofisher.com/primarycells**.

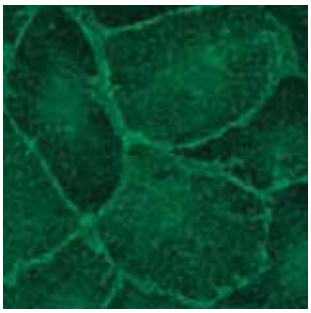
Research applications
Angiogenesis
Dermal research and models
Drug delivery
High-throughput screening
Inflammation
Skin cancer and metastasis
Transdermal studies
Wound healing and burn therapies

Microvascular endothelial cell specifications

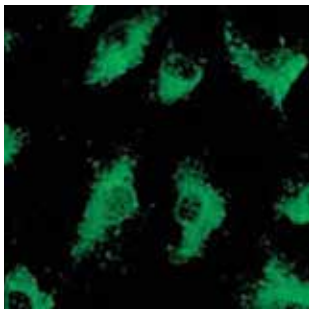
Gibco microvascular endothelial cells are able to grow through at least 16 population doublings, and no potential biological contaminants can be detected. In addition, during the first culture after thawing, the cells take up acetylated-LDL and express von Willebrand factor (vWf), CD31, and CD36 (endothelial cell markers), but not α-actin (a smooth muscle cell marker).



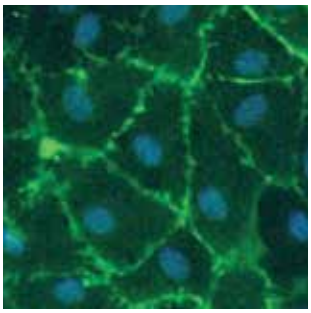
Acetylated-LDL uptake.



Anti-CD36 immunofluorescence.



Anti-vWf immunofluorescence.



Anti-CD31 immunofluorescence, with nuclear counterstain.

Recommended culture systems	
Cells	HMVECad
Basal medium	Medium 131
Growth supplements	MVGS
Reagents	Trypsin/EDTA Solution Gentamicin/amphotericin Trypsin Neutralizer Solution Attachment Factor Protein

Microvascular endothelial cells

Characterization of human microvascular endothelial cells

Each lot of cells is performance tested in our laboratory for viability, growth potential, and for differentiation markers. The cells are also tested for potential biological contaminants (HIV-1, hepatitis B, and hepatitis C viruses, mycoplasmas, bacteria, yeast, and other fungi). To be approved for distribution, the cells must be at least 70% viable upon thawing, each vial must contain at least 500,000 viable cells, cells must be able to grow through at least 16 population doublings, and no potential biological contaminants must be detected. In addition, during the first culture after thawing, the cells must take up dil-Ac-LDL and express von Willebrand factor (vWf), CD31, and CD36 (endothelial cell markers), but not α-actin (a smooth muscle cell marker). Certificates of Analysis are available on our website, or by request.

Human Microvascular Endothelial Cells (HMVEC), neonatal

HMVECnd, cryopreserved

Normal human microvascular endothelial cells isolated from neonatal dermis, cryopreserved at the end of the tertiary culture.

Ordering information

Quantity	Cat. No.
1 vial (>500,000 viable cells)	C-010-5C

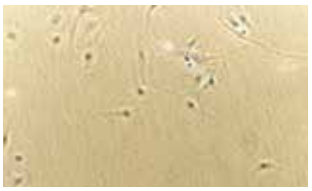
HMVECnd, proliferating*

Quaternary cultures established from cryopreserved cells, grown to approximately 50% confluence in T-flasks using the following medium and supplement combination:

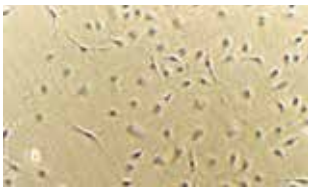
Ordering information

Product	Quantity	Cat. No.
HMVECnd, proliferating culture, prepared in Medium 131 and MVGS	6 x T-25 flasks	C-010-25P

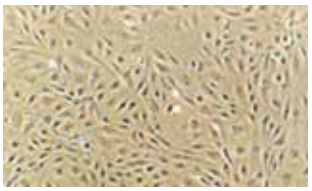
*Setup required. Proliferating cultures are currently available in the US only.



HMVECad, day 1



HMVECad, day 3



HMVECad, day 5

Human Microvascular Endothelial Cells (HMVEC), adult

HMVECad, cryopreserved

Normal human microvascular endothelial cells isolated from adult dermis, cryopreserved at the end of the tertiary culture.

Ordering information

Quantity	Cat. No.
1 vial (>500,000 viable cells)	C-011-5C

HMVECad, proliferating*

Quaternary cultures established from cryopreserved cells, grown to approximately 50% confluence in T-flasks using the following medium and supplement combination:

Ordering information

Product	Quantity	Cat. No.
HMVECad, proliferating culture, prepared in Medium 131 and MVGS	6 x T-25 flasks	C-011-25P-A

*Setup required. Proliferating cultures are currently available in the US only.

Microvascular endothelial media

Basal medium for microvascular endothelial cells

Gibco™ Medium 131 is a sterile, liquid medium for the culture of human microvascular endothelial cells. This basal medium requires the addition of MVGS (Cat. No. S-005-25) prior to use. Medium does not contain antibiotics or antimycotics. Includes one bottle (100 mL) of Gibco™ Attachment Factor Protein (Cat. No. S-006-100).

Ordering information

Quantity	Cat. No.
500 mL	M-131-500

Microvascular endothelial supplements and reagents

Growth supplements for microvascular endothelial cells

Supplements do not contain antibiotics or antimycotics.

Microvascular Growth Supplement (MVGS)

A sterile, concentrated (20X) solution intended for use with Medium 131 to culture human microvascular endothelial cells. Contains fetal bovine serum, basic fibroblast growth factor, epidermal growth factor, heparin, hydrocortisone, and dbcAMP.

Ordering information

Quantity	Cat. No.
25 mL	S-005-25

Attachment Factor Protein

A sterile solution (1X) containing gelatin as an attachment factor (AF). When used to coat culture surfaces, an AF enhances the growth of microvascular endothelial cells.

Ordering information

Quantity	Cat. No.
100 mL	S-006-100

Recommended reagents		
Gentamicin/Amphotericin Solution	10 x 1 mL	R-015-10
Trypsin/EDTA Solution	100 mL	R-001-100
TrypLE Express Enzyme	100 mL	12604-013
Attachment Factor Protein	100 mL	S-006-100

Corneal epithelial cell culture

Corneal culture cell culture overview

We offer a complete Gibco™ system for corneal epithelial cell culture. Our Gibco™ corneal cell culture products have been developed to work together to provide optimal performance. Human corneal epithelial cells (HCECs) are normal corneal epithelial cells isolated from the progenitor-rich limbal region of the eye where the cornea and sclera meet. Limbal tissue is known to be enriched for corneal epithelial progenitor cells. For a complete review of Gibco primary cell technologies and services, visit **thermofisher.com/primarycells**.

Corneal cell specifications

Gibco™ primary HCECs are prepared to provide ≥70% viability upon thawing, with each vial containing sufficient cells to seed ~100 cm^2 of tissue culture surface. Each lot of HCECs undergoes performance testing and is guaranteed to achieve at least 12 population doublings (PD) after thawing when using Gibco™ Keratinocyte Serum-Free Medium (KSFM). Gibco corneal cells stain positively in immunocytochemistry screens for the corneal epithelial markers cytokeratin 15 and p63 alpha.

Research applications	
Cell therapy	Irritancy testing
Drug/compound screening	Ocular research and models
Drug discovery projects	Toxicity testing
Effects of chemical exposure	Wound healing

Corneal cells

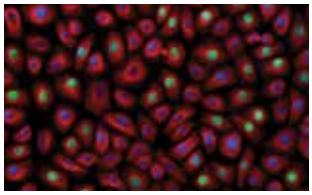
Human Corneal Epithelial Cells (HCEC)

Normal human corneal epithelial cells isolated from the progenitor-rich limbal region of the eye, and cryopreserved at the end of the secondary culture in a medium containing 10% DMSO. Gibco HCECs are ideal for research into corneal biology including: inflammation and wound healing, investigating the effects of chemicals and components used for consumer products, and other studies into ocular function.

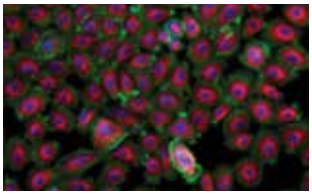
Ordering information

Quantity	Cat. No.
1 vial (>500,000 viable cells)	C-018-5C

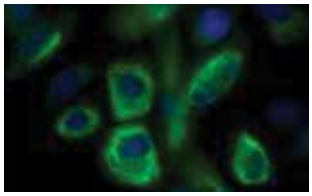
Serum-free culture system	
	Culture environment
	Serum-free culture system
Cells	HCEC
Basal medium	Keratinocyte Serum-Free Medium Kit
Growth supplement	Supplied with Basal Medium Kit
Reagents	TrypLE Express Enzyme
	Defined culture system
Cells	HCEC
Basal medium	Defined Keratinocyte Serum-Free Medium Kit
Growth supplement	Supplied with Basal Medium Kit
Reagents	TrypLE Express Enzyme, Coating Matrix Kit



HCECs imaged using the Invitrogen™ Click-iT™ EdU Alexa Fluor™ 488 Imaging Kit and an anti–α-tubulin antibody with a goat anti-mouse Alexa Fluor™ 555 secondary antibody, and Hoechst 33342.



HCECs labeled with Invitrogen™ Alexa Fluor™ 488 phalloidin and anti–α-tubulin primary antibody and goat anti-mouse Alexa Fluor™ 555 secondary antibody, and counterstained with Invitrogen™ HCS CellMask™ Blue Stain.



HCECs imaged using Invitrogen™ CellLight™ ER-GFP, BacMam 2.0 and Hoechst 33342.

Corneal media and supplements

Keratinocyte-SFM

A sterile, serum-free liquid medium that supports the robust growth of human corneal epithelial cells, keratinocytes, and other types of epithelial cells. It contains L-glutamine and is supplied as a kit that includes aliquots of bovine pituitary extract (BPE) and recombinant epidermal growth factor (rEGF).

Ordering information

Quantity	Cat. No.
500 mL	17005-042

Defined Keratinocyte-SFM

A sterile, defined liquid medium that supports the robust growth of human corneal epithelial cells, keratinocytes, and other types of epithelial cells. Keratinocyte SFM (KSFM) is free of serum and bovine pituitary extract and supplied as a kit that includes single aliquots of growth supplement containing factors that include insulin, EGF, and Fibroblast Growth Factor (FGF) from Bovine Pituitary Extract (Figure 4). For optimal performance, we recommend using defined KSFM in conjunction with Coating Matrix Kit (Cat. No. R-011-K).

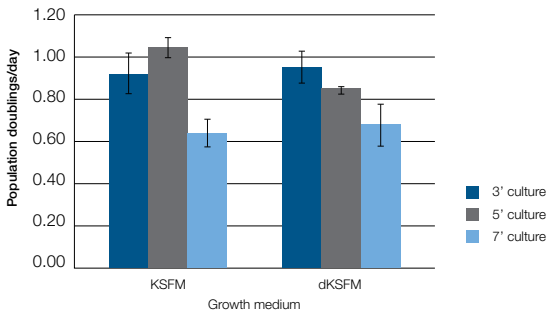


Figure 4. HCEC growth rates. HCECs were thawed and seeded according to product instructions in Keratinocyte SFM (KSFM) or defined Keratinocyte-SFM (dKSFM). Cells were passaged at 90% confluence, and population doublings per culture were calculated. Bars show the mean of triplicate T-25 flasks with standard deviation.

Ordering information

Quantity	Cat. No.
500 mL	10744-019

Recommended reagents		
Cell dissociation		
TrypLE Express Enzyme	500 mL	12604-021
Coating		
Coating Matrix Kit	1 kit	R-011-K
Cryopreservation		
Synth-a-Freeze Cryopreservation Medium	50 mL	A1254201

Cryopreservation

Synth-a-Freeze cryopreservation medium

A defined, protein-free, sterile cryopreservation medium containing 10% DMSO. Suitable for the cryopreservation of all cell types presented in this sourcebook, with the exception of melanocytes.

Ordering information

Quantity	Cat. No.
50 mL	A1254201

Custom primary cells and media

We welcome requests for custom preparations of cell culture products and contract research. Please contact us and we will work with you to develop a solution that meets your research and budgetary needs.

The custom order process is designed on an individual basis, enabling us to tailor the process to suit each request. Once we determine the specifications for the project, we will provide you with a quote for all work and a timeframe for its completion. As always, our technical support and customer service staff are available to assist you every step of the way—from developing the initial specifications to final packaging and delivery.

We have the technical and manufacturing capabilities to produce a wide variety of customized cell culture media and reagents. From slight formulation modifications to complicated engineered-to-order products, custom products are available in both standard and highly specialized packaging configurations. Wherever possible, we can formulate custom media with non–animal-origin components and offer developmental support to help you re-engineer formulations to meet regulatory and performance goals. We offer four distinct formats for media: ready-to-use (1X) liquid media, dry powder media (DPM), liquid media concentrates (LMCs), and Advanced Granulation Technology™ (AGT™) media.

Custom cell culture products and services

- Custom cell isolations and configurations
- Custom medium and supplement formulations
- Cell pellets suitable for RNA isolation and other purposes
- Additional cell characterization and virus testing
- SynerGy™ Selector—online bag design tool

Contact your account manager or technical sales specialist for more details.

Analysis

BacMam technology

Invitrogen™ BacMam technology is based on an insect virus (baculovirus) to help efficiently deliver and express genes in mammalian cells. The baculovirus has been modified to include an expression cassette for transgene expression in mammalian cells.

Benefits include:

- Efficient transduction of mammalian cell lines, including primary cells (fibroblasts, hepatocytes, cardiovascular cells, and epithelial cells) and stem cells (neuronal and mesenchymal cells)
- Safety (nonreplicating in mammalian cells) and lack of observable cytopathic effect
- Frozen storage of pretransduced cells generates assay-ready cells
- Assay development speed (no need to spend time generating a stable cell line)

Go to **thermofisher.com/bacmam**

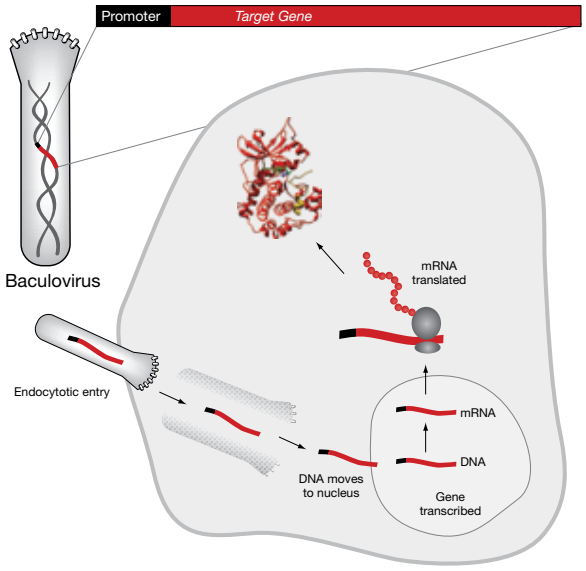


Figure 5. BacMam-mediated gene delivery.

Neon Transfection System

For simple transfection of stem cells, we offer the Invitrogen™ Neon™ Transfection System, a next-generation electroporation technology for highly efficient delivery (~80%) of nucleic acids (plasmid DNA and siRNA) into virtually any animal cell type.

Benefits include:

- The Neon Transfection System has been demonstrated to transfect many difficult-to-transfect cells, including stem cells
- Using a simple optimization experiment, conditions are easily adjusted to maximize delivery efficiency and cell viability

Go to **thermofisher.com/neon**

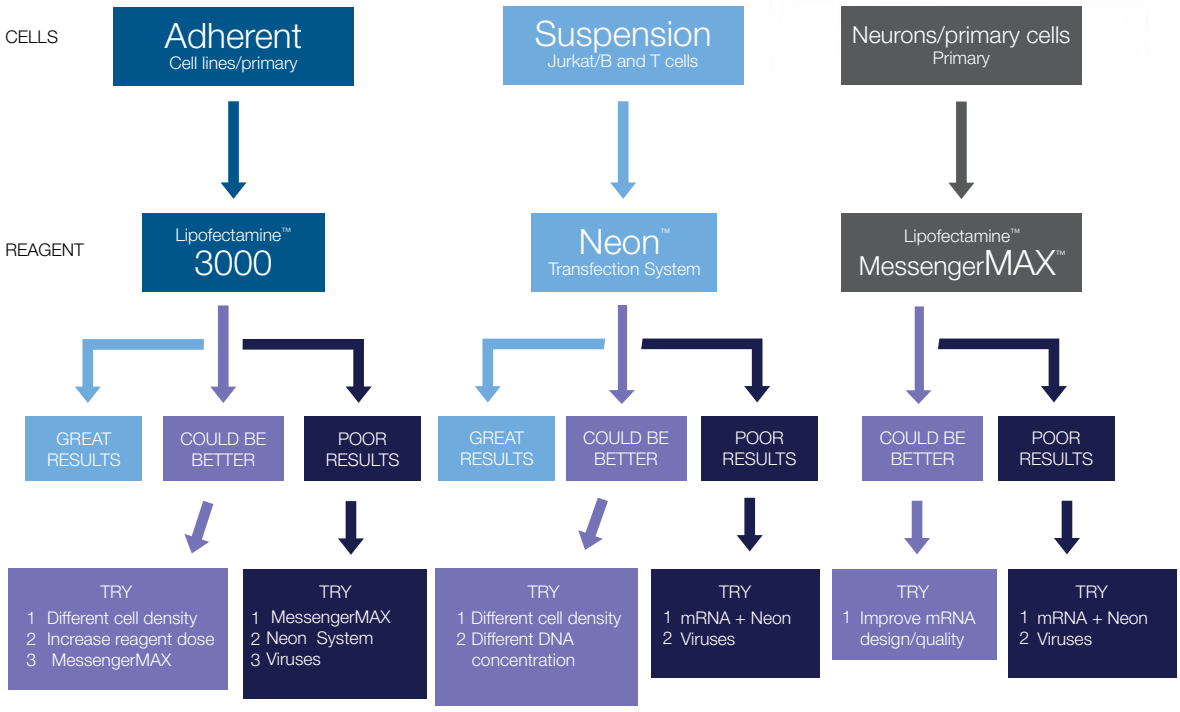
The Neon Transfection System offers breakthrough technology for transfection of primary cells, stem cells, and other difficult-to-transfect cells.

Lipofectamine MessengerMAX Transfection Reagent is your ticket in

Introducing the mRNA transfection reagent with up to 5x the efficiency of DNA reagents in neurons and primary cell types.

- Consistently achieve 60% transfection efficiency in neurons and primary cell types
- Faster protein expression with no risk of genomic integration
- Goes directly to the cytoplasm—great for nondividing or slowly dividing cells
- Up to 10x higher cleavage efficiency using CRISPR mRNA

Go to **thermofisher.com/messengermax**



Lipofectamine 3000 Transfection Reagent

This versatile reagent enables up to 10-fold higher transfection efficiency into difficult cells.

- Superior performance—70% transfection efficiency in difficult cells
- Versatility—highest efficiency into the broadest spectrum of difficult-to-transfect and common cell types
- Gentle with low toxicity—for improved cell viability

Go to **thermofisher.com/3000**

CellLight reagents

Invitrogen™ CellLight™ reagents are fluorescent protein–signal peptide fusions that permit accurate and specific targeting to cellular structures, including the cytoskeleton, for live-cell imaging applications, or for fixed-cell analyses following formaldehyde-based fixation.

Cellular labeling with CellLight reagents employs BacMam technology, which uses a modified insect cell baculovirus coupled with a mammalian promoter as a vehicle to efficiently deliver and express genes in mammalian cells. Unlike expression vectors, BacMam reagents enable titratable and reproducible expression and offer high cotransduction efficiency, enabling multiple BacMam reagents to be used in the same cell.

Go to **thermofisher.com/celllight**

Attune NxT Flow Cytometer

Sensitive acoustic focusing technology with single-cell analysis

Identifying distinct cell types in stem cell research is easily accomplished using the Invitrogen™ Attune™ NxT Flow Cytometer—the first commercially available instrument to give you the power to focus cells into a single line, completely independent of the rate at which the cells flow. The focusing capability of the Attune NxT Flow Cytometer enables rapid rare-event analysis without sacrificing sensitivity. The variable flow rate also allows for optimal peak resolution, even at high sample rates.

Key features:

- Breakthrough acoustic technology focuses cells or beads
- Highest sample delivery rates commercially available
- Automated and user-defined compensation
- Simplified fluorescence compensation
- Countertop instrument—fits on standard lab bench or in laminar flow hood

Go to **thermofisher.com/attune**

Attune NxT Flow Cytometer

Find out more at **thermofisher.com/primarycells**

ThermoFisher SCIENTIFIC