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Application Note

Skin Tissue Model Kit B

Description

The Skin Tissue Model Kit provides the optimal bioinks for encapsulation of dermal and epidermal cells then further conduct functional analysis through immunofluorescence or immunohistochemistry using the provided antibodies.

Package contains:

- GelXA SKIN bioink – 2 x 3 mL
- Collagen type 1 antibody* – 100 µL
- Keratin 10 antibody* – 100 µL
- Elastin antibody * – 100 µL

*Host: Rabbit, Verified Species Reactivity: Human

Storage

The bioinks should be stored between four and eight degrees Celsius. The shelf life of the bioink is two months. The expiration date is stated on the package. Ensure the cartridges are capped prior to storage to prevent drying. Keep the bioink unfrozen – placing the bioink in the freezer risks impairing its printability. Keep the bioink protected from light if transferred from the UV protective cartridge to avoid crosslinking before printing. Work with 3D bioprinters in dark mode. The photoinitiator is sensitive to repeated or prolonged exposure to heat.

The antibodies should be stored at -20°C upon arrival. When stored at -20°C the antibodies are functional for at least twelve months. Note that the antibodies are very stable and can when stored properly be used for longer. The antibodies do not need to be aliquoted prior to storage at -20°C. Since the antibodies are provided in glycerol they will not freeze in the recommended storage temperature and are thus not exposed to freeze/thaw cycles.

Mixing with Cells

It is recommended that the GelXA SKIN is warmed up to 37°C prior to mixing with cells. We recommend mixing the bioink with a high concentration of cells. You can either mix the cells manually or, for larger quantities, use our revolutionary CELLMIXER. The CELLMIXER is designed to simplify the mixing process and enable a homogeneous suspension with an increased cell viability. Please see the *Mixing with Cells Protocol GelXA Series* for more details.

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Bioprinting Tissue Models

Skin tissue models can be generated with bioprinting by layering a dermis compartment followed by an epidermal compartment. See *Printing Skin tissue model Protocol GelXA Series* for guiding and visit our website to download the example codes for bioprinting skin tissue models.

The Skin Tissue Model Kits are compatible with the skin cells of your choice. Dermal fibroblasts and epidermal keratinocytes generate a stable base for the skin tissue model. Use with for example melanocytes to create a photo-sensitive skin tissue model, micro vascular endothelial cells and a VasKit for vascularized skin tissue model or cells from specific donors to make an ethnical specific model.

Crosslinking

Crosslinking of the GelXA SKIN bioprinted constructs can be performed with dual crosslinking capabilities with both the photocuring modules on the BIO X and the $CaCl_2$-containing Crosslinking Agent enhanced with thrombin. Alternatively, crosslinked with only the thrombin containing Crosslinking Agent or photocuring in combination with the thrombin dissolved in Hank's Balanced Salt Solution with calcium and magnesium (HBSS +/+). If using both crosslinking methods, start with photocuring. Recommended to photocure layer by layer. Prior to photocuring, it is recommended that the printbed temperature is reduced to 15°C the print is placed on ice briefly to set the GelXA SKIN. A 5-minute incubation time in Crosslinking Agent is recommended for skin tissue structures in the size of 8 x 8 x 4 mm. After incubation, remove the crosslinking solution, wash with HBSS +/+ or basal cell-culture medium and add the desired cell culture medium.

Histological Analysis

To obtain 5-8 µm sections of the bioprinted construct see *Fixation*, *Embedding* and *Sectioning Protocol GelXA Serie* on the CELLINK website under the Support section. Then follow the *Immunofluorescence staining Protocol GelXA Series* to stain the tissue model with the antibodies. The dilutions for collagen type 1, keratin 10 and elastin antibodies are as follows:

Antibody	Dilution Range	Recommended Dilution
Collagen Type 1	1:50 – 1:200	1:50
Keratin 10	1:200 – 1:800	1:200
Elastin	1:50 – 1:200	1:50

Description of GelXA Bioink

The GelXA based bioinks provide the biological properties of GelMA with printability at a wider range of temperatures such as at room temperature. The GelXA bioinks have dual ionic and photoinduced crosslinking capabilities for better accommodation of cellular sensitivity and allows for tuning of mechanical characteristics of the construct to be more tissue specific. The GelXA bioink line provides minimal optical interference in both

brightfield and fluorescence observations for non-destructive analysis. In histochemistry analysis GelXA will also have minimal background effects, allowing focused investigation of cell-cell and cell-matrix interactions.

Description of Antibodies

The models created with CELLINK's Skin Tissue Model Kits can after treatment with different compounds, such as small molecules or native and non-native substances, be tested for cytotoxicity and cellular response. The antibodies provided with the Skin Kits represents antibodies selective of key factors in a healthy skin tissue. Collagen type 1 is produced by the dermal fibroblasts and is, besides being the main component in the dermis, important for the functionality, structure and firmness of the skin. Keratin 10, also known as cytokeratin 10, is one of the main, early proliferation factors of keratinocytes and present in proliferative keratinocytes closest to the dermis. Elastin is the protein responsible for the crucial elastic properties of the skin, located in the dermis in close connection with the collagens.

Data of Skin Tissue Model Kit

In-house data of skin tissue model's 3D bioprinted with Skin Tissue Model Kit A respectively B and immunofluorescence analyzed with included primary antibodies; collagen type 1, keratin 10 and elastin.

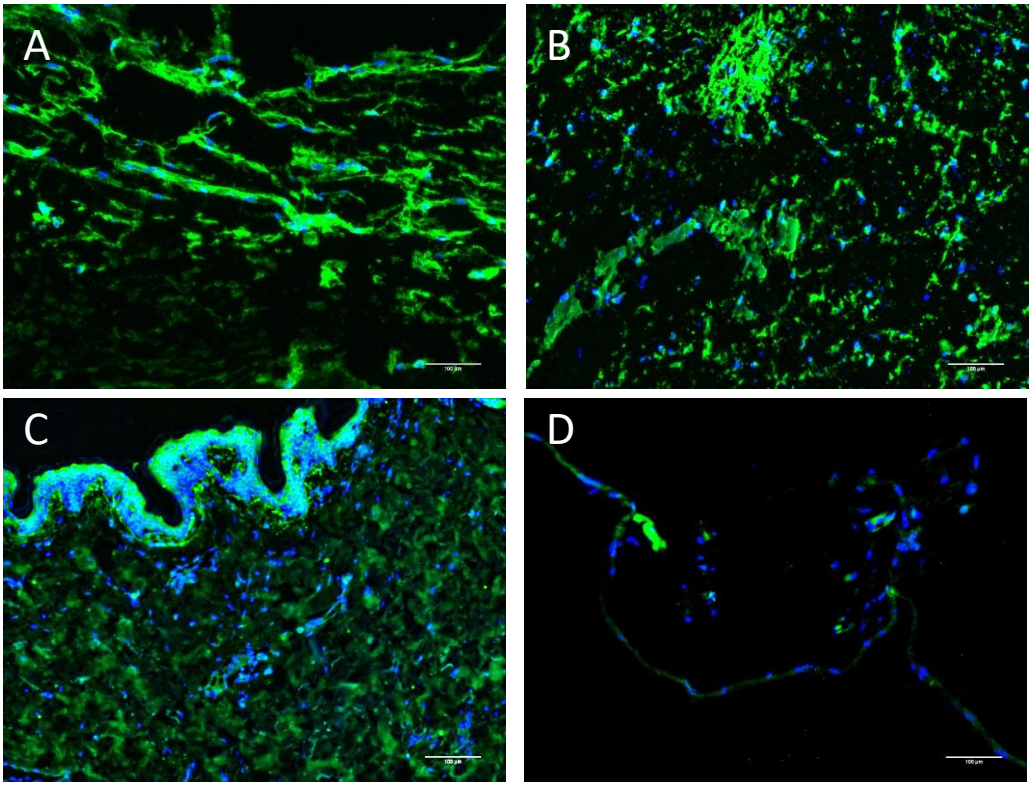

Figure 1. Example of analysis of collagen type 1 expression in 3D bioprinted skin tissue models with GelXA SKIN (Skin Tissue Model Kit B). (A) Skin tissue model, (B) skin tissue model treated with compound and (C) native skin. (D) Control of unspecific antibody

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staining with only secondary antibody applied. Merged images of collagen type 1 expression (1:50 dilution), green, and nuclei stained with DAPI (1:50 dilution), blue. Secondary antibody: Alexa Fluor 488. Magnification: 10X, scale bar: 100 µm.

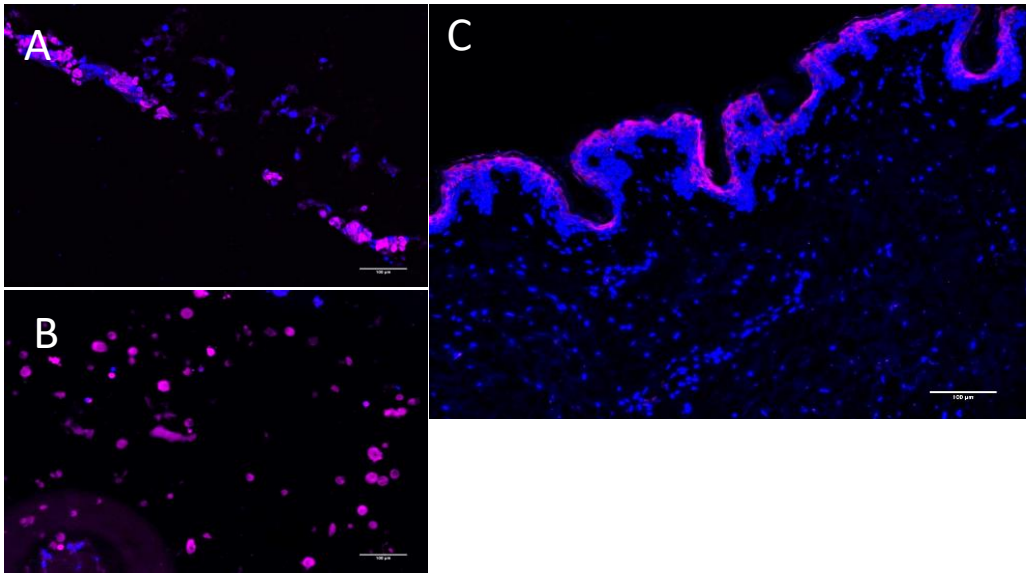

Figure 2. Example of analysis of keratin 10 expression in 3D bioprinted skin tissue models with Skin Tissue Model Kit A and B. (A) Skin tissue model in CELLINK SKIN (Skin Tissue Model Kit A) with seeded keratinocytes. (B) Skin tissue model in GelXA SKIN (Skin Tissue Model Kit B) with embedded keratinocytes and (C) native skin. Merged images of keratin 10 expression (1:200 dilution), magenta, and nuclei stained with DAPI (1:50 dilution), blue. Secondary antibody: Alexa Fluor 488. Magnification: 10X, scale bar: 100 µm.

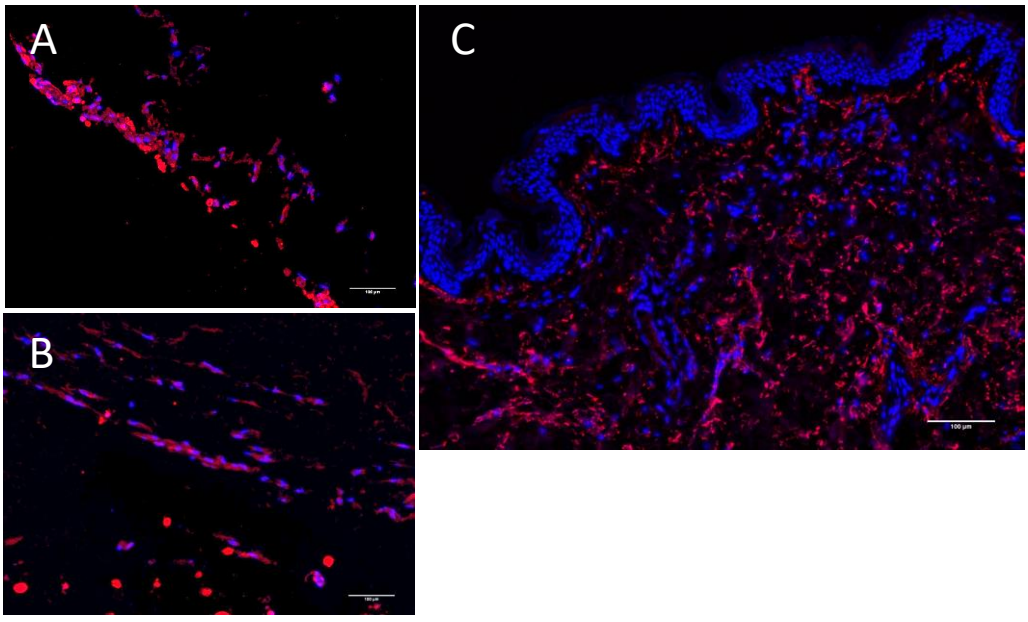

Figure 3. Example of analysis of Elastin expression in 3D bioprinted skin tissue models with Skin Tissue Model Kit A and B. (A) Skin tissue model in CELLINK SKIN (Skin Tissue

Model Kit A) with seeded keratinocytes. (B) Skin tissue model in GelXA SKIN (Skin Tissue Model Kit B) with embedded keratinocytes and (C) native skin. Merged images of elastin expression (1:50 dilution), red, and nuclei stained with DAPI (1:50 dilution), blue. Secondary antibody: Alexa Fluor 488. Magnification: 10X, scale bar: 100 µm.

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