Ref No: APP-KT-021451000C
Approval date: 08-JUN-2019
Author: DN. Version: 2

Application Note

Liver Tissue Model Kit A

Description

The Liver Tissue Model Kit provides the optimal bioinks for encapsulation of hepatocytes and/or stellate cells then further conduct functional analysis through immunofluorescence or immunohistochemistry using the provided antibodies.

Package contains:

- CELLINK LAMININK 111 bioink – 3 mL
- Collagen type I antibody* – 100 µL
- ABCC2 antibody* – 100 µL
- CYP3A43 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.

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

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. See the *Mixing with Cells Protocol CELLINK Series* for more details.

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Bioprinting Tissue Models

Liver tissue models can be generated by bioprinting layer by layer of hepatocytes and/or stellate cells in the desired bioink (CELLINK LAMININK 111). If bioprinting with primary cells, the limited cell numbers will restrict the construct size. Hence, constructs, droplets or discs of 10-50 µL, are adequate for drug screening purposes. Larger constructs can also be generated, especially when layering of cells is desired.

Crosslinking

Crosslinking of the GelXA LAMININK 111 bioprinted constructs is performed with the $CaCl_2$-containing crosslinking solution. After bioprinting, apply enough droplets crosslinking solution to cover the construct. A 30-second to 5-minute incubation time is sufficient for most structures. After incubation, remove the crosslinking solution, wash with Hank's Balanced Salt Solution with calcium and magnesium (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 CELLINK Serie* on the CELLINK website under the Support section. Then follow the *Immunofluorescence staining Protocol CELLINK Series* to stain the tissue model with the antibodies. The dilutions for collagen type I, ABCC2 and CYP3A43 antibody are as follows:

Antibody	Dilution Range	Recommended Dilution
Collagen type I	1:50 – 1:200	1:50
ABCC 2	1:200 – 1:500	1:200
CYP3A43	1:1000 – 1:2500	1:1000

Description of CELLINK LAMININK 111 Bioink

The CELLINK LAMININK 111 is advantageous with stable printability at any temperature in addition to not being sensitive to light. With CELLINK's optimized protocols for staining and analysis the CELLINK LAMININIK 111 bioinks provides a smooth 3D bioprinting process where focus is allowed to be on obtained results. The nanocellulose in the CELLINK bioink provides fibrillar network that can influence the interconnections of the different liver cell types, for example, potentially facilitating the formation of a bile canalicular network or the alignment of stellate cells within a co-culture tissue model.

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Description of Antibodies

Direct functional analysis of the liver tissue model can be conducted with histological analysis through immunofluorescent or immunohistochemistry. The antibodies included in the Liver Tissue Model Kit are for the single or co-culture of hepatocytes and stellate cells in bioprinted constructs. The collagen type I antibody is to evaluate the production of collagen type I by the stellate cells during fibrotic inductions. ABCC2 antibody is to evaluate the transporter multidrug resistance protein 2 (MRP2) in hepatocytes, while the CYP3A43 antibody is for analyzing one the of cytochrome P450 enzymes responsible for metabolism of drug compounds.

Data of Liver Antibodies

The in-house data of immunofluorescence analysis with the included primary antibodies, collagen type I, ABCC2 and CYP3A43, demonstrate optimized usage for different liver cell types and sources.

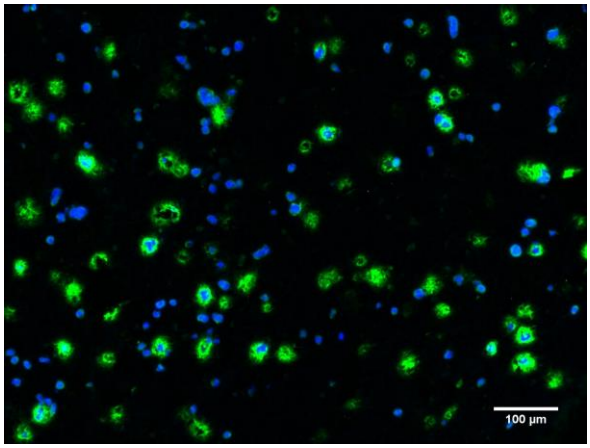

Figure 1. Example collagen type I expression in 3D bioprinted CELLINK LAMININK 111 ladened with co-culture LX2 and HepG2 cells. Merged images of collagen type I expression (1:50 dilution), green, and nuclei stained with DAPI (1:50 dilution), blue. Secondary antibody: Alexa Fluor 488. Magnification: 10X, scale bar: 100 µm.

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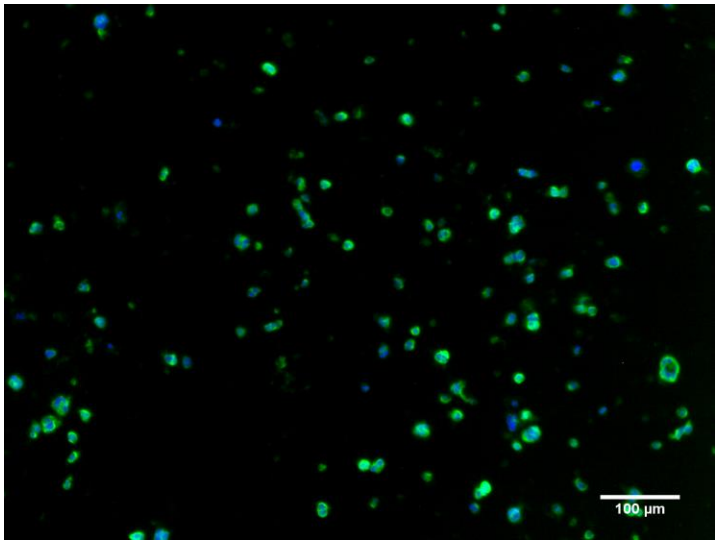

Figure 2. Example of ABCC2 expression in 3D bioprinted CELLINK LAMININK 111 ladened with co-culture LX2 and HepG2 cells. The ABCC2 (MRP2) antibody can be used to stain both hepatocyte HepG2 cell line and primary hepatocytes. Merged images of ABCC2 expression (1:200 dilution), green, and nuclei stained with DAPI (1:50 dilution), blue. Secondary antibody: Alexa Fluor 488. Magnification: 10x, scale bar: 100 µm.

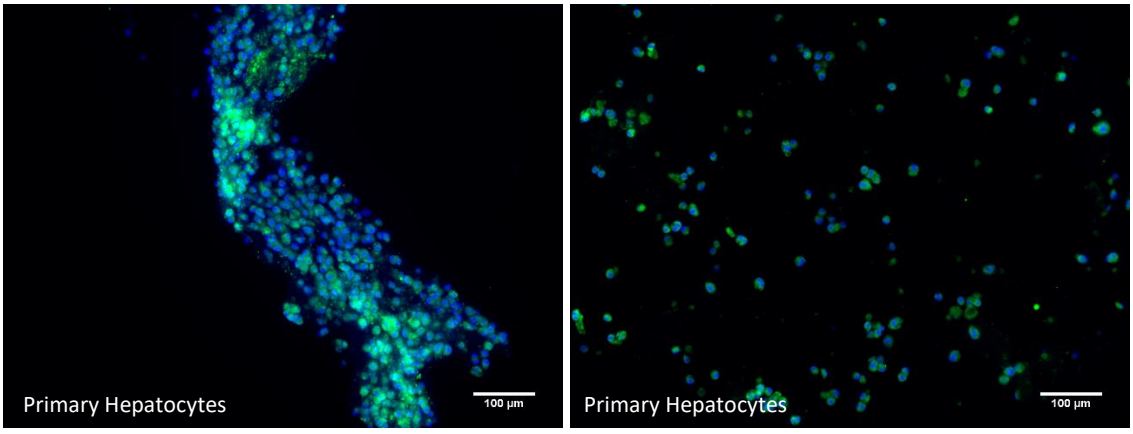

Figure 3. Example of CYP3A43 expression in 3D bioprinted primary hepatocytes as liver tissue models. The CYP3A43 antibody can be used to stain primary hepatocytes in both clusters and single cells. Merged images of CYP3A43 expression (1:1000 dilution), green, and nuclei stained with DAPI (1:50 dilution), blue. Secondary antibody: Alexa Fluor 488. Magnification: 10x, scale bar: 100 µm.

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