# VitroINK<sup>™</sup> Handbook

Ready-to-use bioink for 3D bioprinting

Cat No. INK01, INK02, ISK01, ISK02, IMK00-1, IMK01, IMK02

## Overview

VitroINK is a family of the ready-to-use, xeno-free tunable bioink system that requires no UV, no temperature/pH curing, or chemical cross-linking. The system is ready-to-use at room temperature, neutral in pH, and has excellent visibility after printing and cell culture. Due to the unique shear-thinning and rapid recovery mechanical property, VitroINK can maintain the printed structure without UV or other special curing methods. Adding cell culture medium after printing can further stabilize the printed structure and support cell growth.

Different versions of VitroINK may incorporate multiple biological functional ligands to promote cell attachment, cell-matrix interactions, cell proliferation, motility/migration and differentiation for many different applications.

We strongly suggest using the **VitroINK Mixing Kit–Complete Pack** (Cat No. IMK00-1) to achieve robust mixing of ink and cells. VitroINK 3D Starter Pack (ISK01) and VitroINK RGD Starter Pack (ISK02) contains the bioink and the all the components of the VitroINK Mixing Kit-Complete Pack. Cells and VitroINK ratio can be mixed at 3:1 or 10:1.

## VitroINK versions



| Cat No. | Name                         | Description  |
|---------|------------------------------|--|
| INK01   | VitroINk™ 3D                 | Unmodified bioink for 3D bioprinting                   |
| INK02   | VitroINK <sup>™</sup> RGD    | RGD modified bioink for 3D bioprinting                 |
| ISK01   | VitroINK™ 3D<br>Starter Kit  | VitroINK 3D + Full Mixing Kit<br>(Includes Dispenser)  |
| ISK02   | VitroINK™ RGD<br>Starter Kit | VitroINK RGD + Full Mixing Kit<br>(Includes Dispenser) |

#### **Specifications**

- Xeno-free tunable bioink for 3D bioprinting
- Ready-to-use at room temperature
- No UV, temperature/pH curing, or chemical cross-linking required
- Neutral pH
- Transparent. Excellent visibility after printing and cell culture
- · Pre-mix with cells by using our VitroINK mixing kit
- Ships room temperature. Store at 2-8°C
- Size: 3 mL and 10 mL
- Need a customized VitroINK? Please contact us for a quote.
- Need rheology data of VitroINK with your medium and mixture for publication? Check our data support service .

# VitroINK Mixing Kit – Complete Pack (Cat. No. IMK00-1)

Mixing cells with the bioink is a critical step for 3D bioprinting. Because the differences in viscosity of the cell suspension and bioink, the traditional manual mixing methods create a lot of air bubble and non-uniform mixture, which lead to the unstable printed structure, difficulty for cell observation and affect cell viability.

The VitroINK Mixing Kit-Complete Pack was designed to provide a robust mixing of bioink and cells. Cells can be prepared with VitroINK for a 3:1 mixing ratio using the 3 mL syringe or a 10:1 mixing ratio using the 1 mL syringe. To prepare the bioprinter cartridge, the VitroINK and cell suspension are placed in the mixer and is dispensed through the connector and mixing head. Wait 10-20 minutes for the mixture to stabilize and the cartridge is ready for printing. There is no UV, no temperature/pH curing, or chemical cross-linking for the VitroINK system. Adding cell culture medium to cover the printed structure can further stabilize and support cell growth. The cells are ready for incubation.

The syringe, connector and mixing head are disposable for one-time use. Sterilized replacement mixing kit components (Cat. No. IMK01 or IMK02) can be ordered to ensure optimal mixing results every time.



#### Contents of full VitroINK Mixing Kit – Complete Pack (IMK00-1)

| Catalog No. | Name   | Description   | Specifications   |
|-------------|--|---|--|
| IMK00-1     | VitroINK™ Mixing Kit –<br>Complete Pack              | 3 and 1 mL syringes, dispenser, connector and tubing, mixing head | <ul> <li>Sterilized</li> <li>Ready-to-use</li> <li>Good for 3:1 or 10:1<br/>mixing ratio</li> <li>Ships room temperature.<br/>Store at room temperature</li> </ul> |
| IMK01       | VitroINK™ 3:1 Mixing<br>Component Pack – Single Use  | 1 mL syringes, connector and tubing, mixing head. (no dispenser)  |  |
| ІМК02       | VitroINK™ 10:1 Mixing<br>Component Pack – Single Use | 3 mL syringes, connector and tubing, mixing head. (no dispenser)  |  |

#### Use VitroINK Mixing Kit to prepare cells/ink mixture for 3D Bioprinting



#### Material

- VitrolNK
- VitroINK Mixing Kit Complete Pack (IMK00-1)
- Cell suspension in a culture medium
- Your bioprinter cartridge

### Watch Video Demonstration https://www.thewellbio.com/vitroink-mixing-kit-demo/

#### Protocols



Prepare cell suspension:

Cell concentration:  $10^{6}$ - $10^{7}$  cells/mL. Fill the 3 mL or 1 mL syringe with cells. Fill the syringe with 200-300  $\mu$ l extra volume to fill the empty space of the connector, tubing and mixing head.

#### Step 3

Connect the VitroINK syringe to the female end of the T shape connector (a), the male end of the tubing of the cell syringe to the female end of the T shape connector (b), and the mixing head to the male end of the T shape connector(c).



#### Step 2

Connect the cell syringe to the female end of the connect tubing. Apply pressure to the syringe to fill the empty space of the tubing until a small drop of medium dispenses at the male end of the connect tubing.





#### Step 4

Turn the "OFF" valve of the connector up towards the VitroINK syringe and then gently apply pressure to the **CELL SYRINGE** until a small drop of medium shows up at the end of the mixing head.



#### Step 5

Turn the "OFF" valve of the connector towards the connecting side of cell syringe and then gently apply pressure to the **VITROINK SYRINGE** until a small drop of VitroINK medium comes out from the end of the mixing head.



#### Step 7

**C**onnect the bioprinter cartridge to the mixing head. Turn the "OFF" valve of the connector to open the connection between the VitroINK syringe, cell syringe and mixing head. Gently press the dispenser to mix the VitroINK and cells into the empty cartridge.

Wait 10-20 min for the mixture to become stable. The cartridge is now ready for printing on the bioprinter!



#### Step 6

Clip both the VITROINK SYRINGE and the CELL SYRINGE to the dispenser.



#### Material

- VitrolNK
- Spatula (sterilized)
- Cell suspension in a culture medium
- Petri Dish or small container for mixing
- Your bioprinter cartridge

#### **Protocols**

#### Step 1

Add VitroINK to the petri dish (e.g. 3 mL)



#### Step 4

Remove the piston from the cartridge and use spatula to transfer the mixture to the cartridge

#### Step 2

Add the desired volume of cell suspension to the petri dish (e.g. 1 mL)



#### Step 3

Use the spatula to gently mix the cells and VitroINK. Try to avoid creating air bubbles.





Put the piston back to the cartridge and push the cell/ink mixture to the bottom of the cartridge. Wait 10-20 minutes for the mixture to stabilize. The cartridge is read for printing.





# Growing Cells in New Dimensions

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