PROTOCOL



XENO-FREE INJECTABLE HYDROGEL XENOGRAFT | IN VIVO STUDIES

VitroGel[®] xeno-free hydrogels are excellent for injection and a superior alternative to the animal-based extracellular matrix or plant-based hydrogel for xenograft, targeted delivery, control release, and cell therapy for tissue engineering.



Simple Protocol

Simply mix the hydrogel solution with cells/compounds at room temperature and the hydrogel is ready for injection in 15 minutes. The system is biocompatible without showing a toxic or inflammatory response during the animal safety study.



Maintain an Injectable Status for Hours at Room Temperature or at 37°C

VitroGel has a unique rheological property that can maintain an excellent injectable status for hours after mixing with cells. Researchers do not need to worry about fast gel crosslinking and the need to rush for injection or keeping the solution on ice.

After mixing the hydrogel solution and the cell medium, an injectable soft hydrogel will form. Under the mechanical shearing force such as injection through a syringe, the soft hydrogel performs a gel-sol transition and becomes free-flowing status. After injection, the mechanical strength can rapidly recover with a sol-gel transition and become a hydrogel status again.

TO LEARN MORE: the wellbio.com/xenograft-injection **VIDEO EXAMPLE:** the wellbio.com/xenograft-injection-video



The unique sheer-thinning and rapid recovery properties provide excellent cell retention after injection.



Rev 1.3

For Ready-To-Use Hydrogels Animal Injection—EXAMPLE PROTOCOL

Applies to Cat. No. VHM01, VHM01S, VHM02, VHM02S VHM03, VHM03S, VHM04, VHM04S VHM05, VHM05S, VHM06

All VitroGel hydrogels are injectable and excellent for xenografts. VitroGel is animal component-free, researchers have full control of the supplement/growth factors in the hydrogel-cell mixture by adding the desired compounds to the cell suspension before mixing with hydrogel solution.

MATERIALS AND REAGENTS (AS AN EXAMPLE)

VitroGel[®] Hydrogel Matrix (Cat# VHM01) is used as an example below. Any "Ready-To-Use" hydrogels can be substituted.

- VitroGel Hydrogel Matrix
- Cells or molecular compounds
- PBS or cell culture medium
- Additional supplement (optional)

- Conical tubes (15 mL or 50 mL)
- Micropipette; low retention pipette tips
- Syringe
- 1. Bring VitroGel hydrogel to room temperature.
- 2. Prepare cell suspension in PBS.

Adjust the cell/molecular concentration accordingly to experiment (prepare cell suspension at 2X desired concentration for later mixed with VitroGel for 1X final concentration.

 Mix VitroGel hydrogel with cell suspension at 1:1 (v/v) ratio and gently pipette up and down 5-10 times to mix thoroughly. Example: 1 mL VitroGel hydrogel solution to 1 mL cell suspension in PBS. (The recommended mixing ratios with other solutions are listed in the table below.)

Medium used to prepare cell suspension/drug solution	VitroGel	Cell suspension/drug solution
1X PBS at 1:1 gel/cell ratio (v/v)	1 mL	1 mL
Cell culture medium at 2:1 gel/cell ratio (v/v)	2 mL	1 mL

- 4. Transfer the hydrogel mixture to a syringe. Stabilize the hydrogel mixture either by putting on ice or at 4°C for 5-10 min. Alternatively, stabilize at room temperature for 15 min.
- After stabilization, the hydrogel mixture is ready for injection. The hydrogel mixture can be kept at room temperature during injections. VitroGel has a unique rheological property that can maintain an injectable status for hours after mixing with cells without issues of needle clogging.

Optional for step 2: Adding 30-50% FBS (or your key supplement) or 3-5% BSA/HSA to the cell suspension before mixing with the hydrogel can boost the cell growth after injection.

VIDEO EXAMPLE: thewellbio.com/xenograft-injection-video

Your use of this product is subject to our LIMITED USE LICENSE, LIMITED WARRANTY AND TERMS OF SALE AGREEMENT which can be found at https://www.thewellbio.com/ terms-and-conditions. If your use is not permitted by our Limited Use License, please contact us for authorization of your use. If you do not agree to the terms of our Limited Warranty and our Terms of Sale should return the product in acceptable conditions to the seller for a refund.

For research use only



Page: 2

For High Concentration Hydrogels Animal Injection—EXAMPLE PROTOCOL

All VitroGel hydrogels are injectable and excellent for xenografts. VitroGel is animal component-free, researchers have full control of the supplement/growth factors in the hydrogel-cell mixture by adding the desired compounds to the cell suspension before mixing with hydrogel solution.

MATERIALS AND REAGENTS (AS AN EXAMPLE)

VitroGel[®] MMP (Cat# TWG010) is used as an example below. Any "High Concentration" hydrogels can be substituted.

- VitroGel MMP High Concentration Kit
- Cells or molecular compounds
- PBS or cell culture medium
- Additional supplement (optional)

- Conical tubes (15 mL or 50 mL)
- Micropipette; low retention pipette tips
- Syringe
- 1. Bring VitroGel hydrogel to room temperature.
- Prepare cell suspension or drug solution in cell PBS (1X).
 (Can also use cell culture medium or VitroGel Dilution Solution to sutitute the PBS).
 - Adjust the cell seeding concentration according to your experiment.
 - If you use VitroGel for molecular compounds injection, prepare the compounds in buffer at 3-5X of the desired concentration. The compounds solution then can mix with VitroGel hydrogel solution to get 1X final concentration in step 3.
- 3. Adjust the hydrogel concentration accordingly and then gently mix the adjusted VitroGel solution with cell suspension (drug solution) without introducing bubbles. The recommended ratios of different hydrogel dilutions and mixing are listed in the table below.

Type of medium to prepare cell suspension/ drug solution	Dilution Ratio	VitroGel	Dilution Solution	Cell Suspension/ Drug Solution	Waiting time for hydrogel stabilization	
1X PBS	1:0	2 mL	0 mL	2 mL	- 5-15 min	
	1:1	2 mL	2 mL	4 mL		
	1:2	1 mL	2 mL	3 mL		
	1:3	1 mL	3 mL	4 mL		
Cell culture medium	1:0	2 mL	0 mL	500 μL	- 5-15 min	
	1:1	2 mL	2 mL	1 mL		
	1:2	2 mL	4 mL	1.5 mL		
	1:3	1 mL	3 mL	1 mL		



Page: 3

For High Concentration Hydrogels Animal Injection—EXAMPLE PROTOCOL

CONTINUED

- 4. Transfer the hydrogel mixture to a syringe. Stabilize the hydrogel mixture either by putting on ice or at 4°C for 5-10 min. Alternatively, stabilize at room temperature for 15 min.
- 5. After stabilization, the hydrogel mixture is ready for injection. The hydrogel mixture can be kept at room temperature during injections. VitroGel has a unique rheological property that can maintain an injectable status for hours after mixing with cells without issues of needle clogging.

Optional for step 2: Adding 30-50% FBS (or your key supplement) or 3-5% BSA/HSA to the cell suspension before mixing with the hydrogel can boost the cell growth after injection.

VIDEO EXAMPLE: thewellbio.com/xenograft-injection-video

Your use of this product is subject to our LIMITED USE LICENSE, LIMITED WARRANTY AND TERMS OF SALE AGREEMENT which can be found at https://www.thewellbio.com/ terms-and-conditions. If your use is not permitted by our Limited Use License, please contact us for authorization of your use. If you do not agree to the terms of our Limited Warranty and our Terms of Sale should return the product in acceptable conditions to the seller for a refund.

