

## FAQ document - Xenograft

**1. What is the injection volume of VitroGel?**

Most customers working with mice use the injection volume of 100-200  $\mu\text{L}$  (depending on cell type). For rat models, the injection volume is about 500  $\mu\text{L}$ .

**2. What is the recommended number of cells for VitroGel injection?**

The typical implanting cell number is between 1 to  $10 \times 10^6$  cells according to different cell types. The cell suspension can be prepared in PBS to obtain  $2\text{-}20 \times 10^7$  cells per mL and then be mixed with VitroGel at 1:1 ratio (v/v). The cell density can be adjusted based on the inoculation volume (e.g., 100 or 200  $\mu\text{L}$ ) and the mixing ratio between VitroGel and cell suspension. Please check user protocols for different mixing ratios.

**3. Do I need to keep the VitroGel-cell mixture in an ice bucket?**

Unlike Matrigel, VitroGel is ready-to-use and can mix with cell suspension at room temperature. After hydrogel and cell suspension is mixed and transferred to a syringe, you can put the mixture on ice, or 4 degrees Celsius, for 5-10 minutes to accelerate the injectable hydrogel formation to ensure the homogenous cell suspension. After that, the hydrogel can be kept at room temperature for long-term injection. If you don't want the cold temperature, simply put the hydrogel-cell mixture at room temperature for 15 minutes for gel stabilization before injection. VitroGel has a unique shear-thinning and rapid recovery property, which can maintain a long-term injectable status (hours) and excellent cell retention after injection without the needle clogging issue (Please check VitroGel Xenograft Application webpage for more details: <https://www.thewellbio.com/applications/in-vivo/xenograft-pdx-cdx/>).

**4. How long does the VitroGel-Cell mixture retain its injectable status compared to Matrigel?**

As opposed to Matrigel, which has a fast solidification process when the temperature is higher than 10 degrees Celsius, the VitroGel-cell mixture forms a soft injectable hydrogel. The hydrogel has a unique shear-thinning and rapid recovery property to retain its injectable status for months (unless more culture is not added or for as long as the cells survive).

**5. After injection with VitroGel, is there a worry of cells moving in the host? How efficient is cell retention?**

After injection, the sol-gel transition happens immediately, which rebuilds the mechanical strength of the hydrogel and holds cells within the hydrogel matrix. Hence, our hydrogel system has excellent cell retention after injection.

**6. Do I have to add the serum, growth factors, or supplements to the cell suspension before mixing with VitroGel?**

No. Adding the supplement to the hydrogel matrix is not required. For most xenograft applications, preparing cells in PBS solution works excellently with VitroGel. On the other hand, VitroGel is animal-origin free, which gives the flexibility for researchers to manipulate the supplement in a gel-cell mixture if needed.

**7. If I want to add supplements to boost cell growth, what are the common growth factors or components used?**

Growth factors added depend on your application and system. The common ones include BSA, HSA, serum replacement, etc. If serum supplement is not an issue of your project, you can also add FBS to give the system an extra push to see promising cell growth.

**8. Does the addition of serum and other cytokines at a high concentration to the cell suspension before mixing with VitroGel lead to bubble formation?**

There is no bubble problem due to the low viscosity of the VitroGel. There is an option to warm the medium and hydrogel solution before mixing them, as this can help reduce the viscosity.

**9. Can VitroGel be used for injection in mice at different sites?**

Yes, VitroGel allows for different injection sites like subcutaneous, orthotopic, IP, etc. Some injections that are difficult with the animal-based matrix can smoothly perform with VitroGel.

**10. What is the tumor formation rate and growth kinetic in VitroGel?**

VitroGel performs extremely well on tumor formation. The success of tumor formation rate is almost 100% for typical cell types and over 70% even for some difficult cell types. In most cell types, the tumor growth kinetics in VitroGel are faster or equal to Matrigel.

**11. Is there data on the successful cell types that have worked with VitroGel, specifically for xenograft?**

Yes, you can check the successful cell types on our website (<https://www.thewellbio.com/applications/in-vivo/xenograft-pdx-cdx/>). This list is expanding. If you need to check the most updated successful cell-type table, please contact [support@thewellbio.com](mailto:support@thewellbio.com).

**12. Is there any update on the protocol front for injection using VitroGel?**

Here is the link to the updated xenograft webpage that includes protocols, videos, and relevant data: <https://www.thewellbio.com/applications/in-vivo/xenograft-pdx-cdx/>