FOR FIRST TIME USERS OF THE VITROGEL SYSTEM, PLEASE READ THE FOLLOWING NOTES BEFORE USING THE PRODUCTS

Since different cell types prefer different tissue-specific microenvironments, hydrogel conditions need to be optimized for different cell types and culture media in order to get the best results out of the VitroGel system. For first-time users, an initial test of cell growth in a gradient of hydrogel concentrations is highly recommended. Please use the following steps to setup a gradient of hydrogel concentrations.

Dilute the hydrogel solution:

The ready-to-use VitroGel Dilution Solution is recommend for preparing different hydrogel concentration.

*Users can prepare their own hydrogel dilution solution. Mix 500 mL 1X PBS (without calcium or magnesium) with 500 mL DI water for 0.5X PBS (optional: add 5 wt% sucrose to supplement the osmolarity of the dilution solution).

1. Directly mix VitroGel with the VitroGel Dilution Solution in ratios of 1:0, 1:1, 1:2, 1:3 (VitroGel: VitroGel Dilution Solution, v/v) at room temperature.
2. Mix 4 mL diluted VitroGel from step 1 with 1 mL cell culture medium (with or without cells, keep the mixing ratio at 4:1 (v/v)).
   IMPORTANT NOTE: Please read the "How To Prepare The Cell Suspension" below.
3. Transfer the hydrogel mixture to a well plate and wait 10-20 min at room temperature for a soft gel formation.
4. After soft gel formation, carefully add cell culture medium to cover the hydrogel.

TABLE 1. Volumes of solution/medium for different hydrogel dilutions

Dilution Ratio	VitroGel	VitroGel Dilution Solution	Cell Medium for Mixing
1:0	4 mL	0 mL	1 mL
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

How To Prepare Cell Suspension

If cells cultured in complete cell culture medium, which is supplement with 10% FBS or other critical supplement, please prepare the cell suspension using the following methods before mixing it with hydrogel solution.

1. Prepare the cell suspension with 2X concentration (e.g. 100K), and mix with 100% FBS at 1:1 (v/v) ratio to get 1X cell suspension (50K) with 50% FBS.

2. Mix the diluted hydrogel solution with the cell suspension from above at 4:1 (v/v) ratio to get the final cells in the hydrogel at 10K with 10% FBS supplement.

NOTE 1:

- The VitroGel Dilution Solution will slowly initialize the hydrogel formation, therefore prepare **FRESH** diluted VitroGel and use immediately to mix with cell culture medium.
- After mixing with cell culture medium, immediately transfer the mixture to the tissue culture plate.
  Note: If you have multiple samples with different hydrogel conditions or cell types to prepare, transferring mixture of sample 1 to the tissue culture plate before mixing the hydrogel with cell culture medium for sample 2.
- Mixing VitroGel with 1X PBS would form a soft hydrogel, which can be use for 2D coating or preparing an injectable hydrogel. Using 1X PBS for dilution at 1:2 to 1:4 ratio, might cause the non-uniform hydrogel formation.

NOTE 2: Adjusting The Hydrogel Formation Time

- If VitroGel needs to be diluted more than 1:3 ratio, a longer waiting time (20-30 min) may be needed for soft gel formation. Using a higher volume of cell culture medium for mixing would help to accelerate the process of hydrogel formation.
- If the hydrogel solidifies too fast after mixing with culture medium (showing as small solid gel chunk), adjust the mixing ratio by using less cell culture medium. For example, if mixing 4 mL diluted hydrogel solution with 1 mL cell culture medium lead to the solid gel chuck (particles), then mixing 4 mL diluted hydrogel solution with 0.5-0.8 mL cell culture medium would help to solve the issue.
- On the other hand, if the hydrogel formation is too slow, which may happen when using low hydrogel concentration at 1:3 or 1:4 dilution or using cell culture medium with very low ionic concentration, adjust the mixing ratio by using more cell culture medium. For example, if mixing 4 mL diluted hydrogel solution with 1 mL cell culture medium lead to a slow hydrogel formation, then mixing 4 mL diluted hydrogel solution with 1.5-4 mL cell culture medium would help to solve the issue.