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

As different cell types prefer different tissue-appropriate microenvironment, to get the best results out of VitroGel system, the hydrogel conditions need to be optimized for different cell types and culture media. For first time users, the 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. You can choose to use PBS or DI water to make the hydrogel dilution.

OPTION 1. Use PBS to dilute the hydrogel solution:
(We recommend using 0.5X PBS)

1. Directly mix the hydrogel solution with PBS at 1:0, 1:1, 1:2, 1:3, 1:4 (hydrogel solution : PBS, v/v) ratio at room temperature.
2. Mix the diluted hydrogel solution with cell culture medium (w/ or w/o cells) according the Table 1 below. (The mixing ratio is adjusted according to the concentration of inorganic salts of the cell culture medium, especially [Ca2+]).

TABLE 1. Mixing ratios of dilution hydrogel solution with cell culture medium with different Ca2+ concentration (using 0.5X PBS dilution)

[Ca2+] in culture medium (mg/L)	Volume of diluted hydrogel solution (µL)	Volume of cell culture medium (µL)	Examples
200	400	100	DMEM, EMEM, MEM-Alpha
150	200	100	Leibovitz L-15, MEM (Hanks'), DMEM/F-12, McCoy`s 5A
100	100	100	RPMI 1640

Notes:

- PBS will initialize the hydrogel formation, so prepare **FRESH** the diluted hydrogel solution and use immediately to mix with cell culture medium after diluting with PBS.
- Mixing hydrogel solution with 1X PBS would form a soft hydrogel, which can be use for 2D coating or prepare injectable hydrogel. Using 1X PBS for dilution at 1:2 to 1:4 ratio, might cause the non-uniform hydrogel formation.
- For medium having [Ca2+] lower than 150 mg/mL, we suggest the concentration of the hydrogel solution to be not lower than 1:2 dilution.
- For more diluted hydrogel solution (e.g. 1:3 or 1:4 dilution), use a higher volume of cell culture medium 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), please adjust the mixing ratio by using less cell culture medium. For

example, if mixing 1 mL diluted hydrogel solution with 1 mL cell culture medium lead to the solid gel chuck (particles), then mixing 1 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 (normally happens when using low hydrogel concentration at 1:3 or 1:4 dilution). 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 1.5-3 mL cell culture medium would help to solve the issue.

OPTION 2. Use DI water to dilute the hydrogel solution:

1. Directly mix the hydrogel solution with DI at 1:0, 1:1, 1:2, 1:3, 1:4 (hydrogel solution: DI water, v/v) ratio at room temperature.
2. Mix the diluted hydrogel solution with cell culture medium (w/ or w/o cells) according the Table 2 below. (The mixing ratio is adjusted according to the concentration of inorganic salts of the cell culture medium, especially [Ca2+]. For the same culture medium, the mixing ratio need to be adjusted for different hydrogel dilution to ensure the hydrogel formation within a reasonable time)

TABLE 2. Mixing ratios of dilution hydrogel solution with cell culture medium with different Ca2+ concentration (using DI water dilution)

[Ca2+] in culture medium (mg/L)	Dilution of hydrogel solution with DI water	Volume of diluted hydrogel solution (µL)	Volume of cell culture medium (µL)	Examples
200	1:0, 1:1	400	100	DMEM, EMEM, MEM-Alpha
	1:2	300	100	
	1:3	300 or 200	100	
	1:4	100	100 to 300	
150	1:0, 1:1	200	100	Leibovitz L-15, MEM (Hanks'), DMEM/F-12, McCoy`s 5A
	1:2	100	100	
100	1:0, 1:1, 1:2	100	100	RPMI 1640

Note:

- Prepare **FRESH** the diluted hydrogel solution for each use.
- By using the DI water to dilute the hydrogel solution, the mixing ratio of the diluted hydrogel solution and cell culture medium need to adjust according to different dilution.
- For medium has [Ca2+] lower than 150 mg/mL, we suggest the concentration of the hydrogel solution not to be lower than 1:2 dilution.

- For more diluted hydrogel solution (e.g. 1:3 or 1:4 dilution), the volume of cell culture medium can be adjusted to higher than the recommend volume of Table 2 (page 6) to accelerate the process of hydrogel formation.
- If the hydrogel solidifies too fast after mixing with culture medium (showing as small solid gel chunk), please adjust the mixing ratio by using less cell culture medium. For example, if mixing 1 mL diluted hydrogel solution with 1 mL cell culture medium lead to the solid gel chuck (particles), then mixing 1 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 (normally happens when use low hydrogel concentration at 1:3 or 1:4 dilution). 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 1.5-3 mL cell culture medium would help to solve the issue.