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Instruction Guide

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Instruction Guide for use of Syntrix-P Kit

Syntrix-P is a synthetic matrix that can be used to create defined 3D micro-environments for a variety of cell-based assays. Syntrix-P has been functionalized with oligoethyleneglycol moieties, thereby presenting a low-adhesion surface to cells. In the presence of cell culture medium, Syntrix-P self-assembles into a hydrogel that exhibits a nanofiber structure, resembling the natural extracellular matrix. The resulting hydrogel has been found to support various cell lines (e.g. 3T3, HepG2, MDCK, and CaCo-2) and primary cells (e.g. HUVEC, hADSC and hESC).

Kit content

Syntrix-P Kit	Amount
Syntrix-P (Solution A)*	2 x 1.65 mL
Hyaluronic acid (Solution B)*	1 x 1.5 mL

**Store at -20°C*

General considerations

- Check carefully for any precipitation after thawing solution A. If necessary, redissolve any precipitation by pipetting up and down several times.

- Aliquot solution A in appropriate volumes after thawing for the first time to avoid multiple freeze thawing cycles. In addition, keep the time solution A is at room temperature to a minimum, i.e. put it back at -20 °C immediately after performing experiments.

- Syntrix-P can be used in a concentration range of 3-9 mg/ml final matrix concentration. The volumes in Table 1 lead to a final matrix concentration of 5 mg/mL, providing a good starting point for optimization for your specific application. As different cell types may require different concentrations, we refer to the document **Protocols** for the required volumes for other concentrations

- When used at a concentration of 5 mg/ml and a volume of 60 µl per well in a 96-well plate 1 Syntrix-P kit provides enough matrix for ~2.5 96-well plates.

- Syntrix-P solutions are acidic (pH 2-2.5). Right after encapsulation, the gel pH will be around 6.5-7.5. The pH will subsequently equilibrate with added medium.

- Gelation of Syntrix-P is triggered through neutralization by the buffer present in cell culture media and starts **immediately** upon mixing. Therefore, it is very important that mixing of "Cell suspension + Solution B" with "Syntrix-P" (Solution A) is performed quickly but carefully (avoid introduction of air bubbles). We recommend to start plating the matrix **directly upon mixing**, as disruption of partially formed gels will negatively influence the final gel integrity.

- Cells have been cultured in Syntrix-P in concentrations ranging from $4*10^4$ - $2.5*10^7$ cells/mL. Titration is needed to determine the optimal cell-density for your experiment. A good starting point for high throughput experiments is to plate your cells at a density of $4*10^4$ – $2*10^5$ cells per mL of gel.

- Matrices prepared using cell culture media with Phenol Red pH indicator will change color from salmon colored to light pink over a period of 0.5-2 hours after plating. Syntrix-P is compatible with all standard culture media.

- It is important to minimize air bubble formation, as the presence of air bubbles in the final gels interferes with their attachment to the wells.

- To fill larger numbers of wells it is recommended to use a multi-channel pipette.

- Syntrix-P matrices increase in transparency over time. For obtaining a higher optical transparency use lower gel volumes as this will reduce the light absorbance of the gel.

- Syntrix-P is a fully defined synthetic matrix that, in contrast to basal membrane extracts, does not contain soluble growth factors or cytokines. To achieve optimal cell growth and differentiation, addition of bioactive molecules (e.g. growth factors, cytokines, etc.) to the culture medium may be required.

3D Cell Encapsulation in Syntrix-P (Figure 1)

1. Let Solutions A and B come to room temperature (**do NOT heat to 37 °C, as this will influence the structural integrity and homogeneity of the gels**). Add the appropriate amount of Solution A (see Table 1) to a sterile fresh tube.

2. Prepare your cell suspension in your culture medium of choice at 1.4 times the desired final cell density (see Table 1). Slowly pipet up solution B (highly viscous) and add to the

cell suspension. Mix well by pipetting up and down 10-20x avoiding the formation of air bubbles. **It is recommended to prepare 1.2 times the required volume**.

Table 1: Recommendations for use of Syntrix-P

Format					**96-wells plate**				**384-wells plate**
A : B : cell suspension*					1 : 0.35 : 3.2				1 : 0.35 : 3.2
Recommended gelation time					30 min.				30 min.
Recommended amount of final gel per well					40-80 µL**				15-50 µL
Recommended amount of medium on gel					2 x gel volume				2 x gel volume
96-well plate (60µl / well)					**384-well plate (25µl / well)**				
# of wells	**Solution A**	**Solution B**	**Cells + medium**	**Total**	**# of wells**	**Solution A**	**Solution B**	**Cells + medium**	**Total**
8 wells	127µl	45µl	408µl	580µl	**16 wells**	105µl	37µl	338µl	480µl
16 wells	233µl	82µl	745µl	1060µl	**32 wells**	211µl	74µl	675µl	960µl
96 wells	1288µl	451µl	4121µl	5860µl	**384 wells**	2532µl	886µl	8102µl	11520µl

** Use 1.4x the desired cell density.*

***When using volumes of 50 µl or lower, wells should be pre-wetted with culture medium.*

Note: smaller amounts of gel (35µl 96-well; 15µl 384-well) can be used as a surface coating in order to seed cells on top of the gel.

3. Add the "Cell Suspension + Solution B" premix (prepared in step 2) **all at once into** Solution A and mix **immediately** by pipetting the solution **quickly but carefully** up and down for two times. While mixing avoid pressing the pipet through the final resistance as this might introduce air bubbles in the mixture.

   Attention!
   Do not mix by adding solution A to the "Cell Suspension + Solution B" premix as this will cause incomplete mixing and non-transparent fragile gels.

4. Start pipetting the Gel – Cell Suspension **directly** into the microplate. Pipet up slowly and add to the center of the well. Avoid taking in air bubbles (see Table 1 for recommended volumes per plate format). Ideally dispensing into the plates should be finished within 3 min after mixing in order to have optimal gel structural and optical properties, and to avoid start of gelation into the tips.

5. Leave the suspension for 30 minutes (see Table 1) at Room Temperature to become a gel without moving the well plate. Gently add culture medium (see Table 1) by pipetting via the wall of the well and place the gels in a 37°C incubator for cell culture.

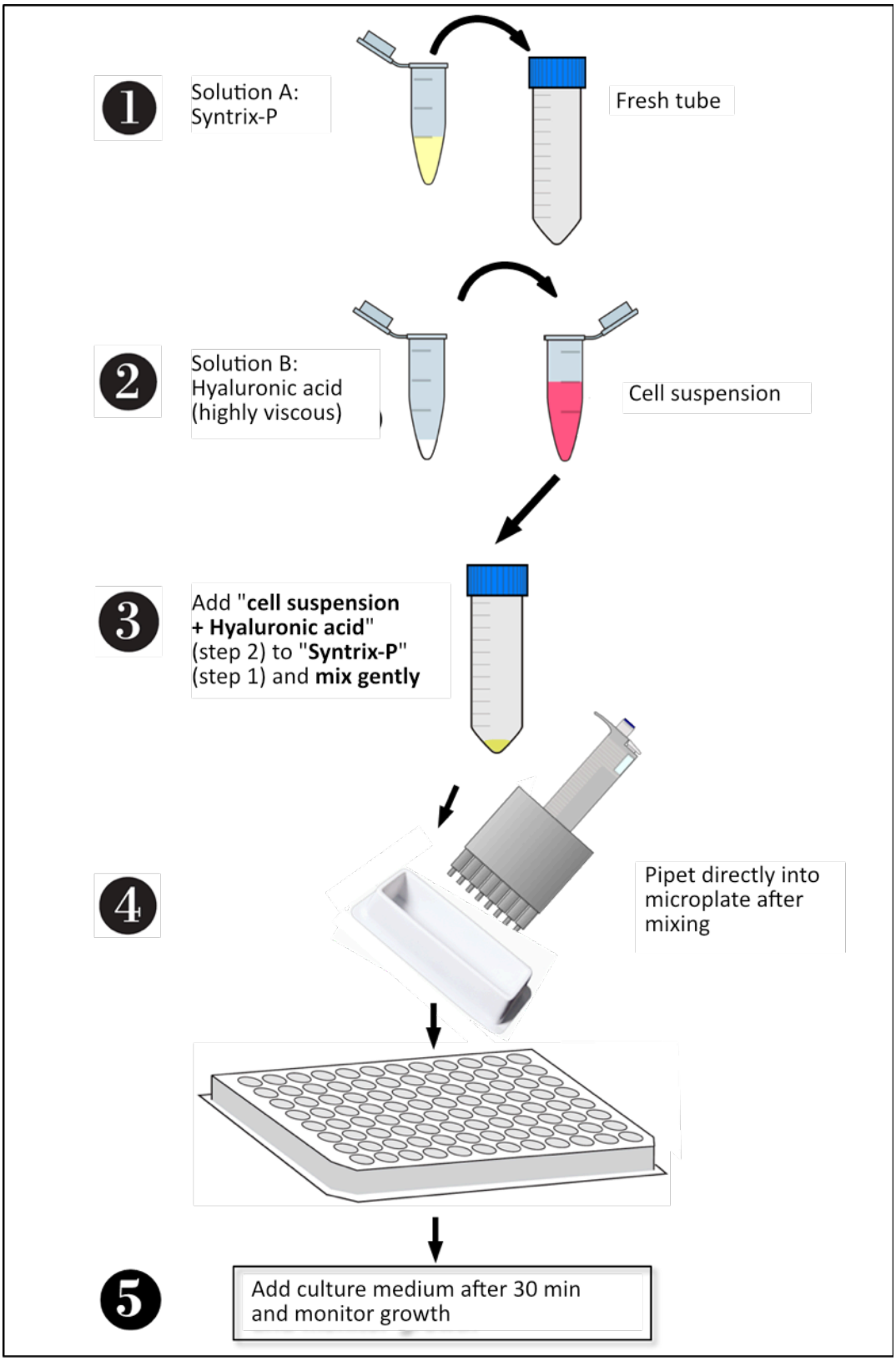

Figure 1: Encapsulation of cells into Syntrix-P