User Protocol: Hydration of CollaFibR™ Scaffolds for 3D Cell Culture

Product storage and handleability:

- This product is sterile upon delivery.
- Store the product at room temperature, away from light and moisture.

Equipment and materials needed:

- Humidified incubator
- Biological safety cabinet (BSC)
- Water bath
- Well plate containing CollaFibR[™] inserts
- Sterile dPBS
- Cell suspension in appropriate culture medium

Preparing CollaFibR™ inserts for cell culture:

1. Place the well plate containing the CollaFibR[™] inserts in the humidified incubator overnight prior to use.

2. Warm the dPBS to at least room temperature before beginning the hydration process.

3. Working inside the BSC, gently pipette 500 μL of dPBS onto the sloped side of the inner wall of the insert ring.

4. Incubate each insert in dPBS for a total of 30 minutes at room temperature.

- 5. After 30 minutes, replace the dPBS with fresh dPBS as follows.
 - a. Tilt the well plate toward you and gently remove 250 µL of dPBS from each insert.
 - b. Rest the well plate flat and gently add 250 μ L of fresh dPBS onto the sloped side of the inner wall of the insert ring.
 - c. Repeat steps a & b once more.
- 6. Remove the dPBS and add cells as follows.
 - a. Tilt the well plate toward you and gently remove the 500 μL of dPBS.
 - b. Rest the well plate flat and gently pipette 500 μ L of cell suspension or media control onto the slopped side of the inner wall of the insert ring.



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