Model: SK-5

Net Mold Starter Kit with Sphere Ring 20

Fabricate 3D Tissue up to 14x14x1mm size!

Process Flow

1: Prepare Cell Aggregate
   - Fill in by Pipette.
   - >150um
   - 2 days

2: Fill in to Mold Cavity
   - Shaker in Incubator
   - 5-10 days

3: Maturation
   - Slide out Nets.
**Data 1**

**Sphere Ring 20**

Sphere Ring 20 (φ90×20 mm)

Cell: TkDN4-M (human iPS cell)
Medium: Essential8 (Thermo Fisher)
Seeding density: 4.0×10⁶ cells/bag

[Data: University of Tokyo, Sakai-Lab]

- Volume: 20 ml
- Agitation: 50 rpm
- Culture: 4 Days

**Cell Block made with Human Dermal Fibroblasts.**

**Data 2**

**Cell**: Human Fibroblast

Medium: FKCM201T (Fukoku Co., Ltd)
Seeding density: 1.0×10⁷ cells/bag

- Volume: 20 ml
- Agitation: 45 rpm
- Culture: 3 Days

**Manufacturer**: FUKOKU CO., LTD.

**Net Mold NM14-1**

Spheroids fed into 14x14x1mm cavity

**Block Size**

2x2x1mm
4x4x1mm
6x6x1mm
15x15x1mm

(Inner needle removed)

**HE Stained.**

Cell survives inside after 15 days.

**14x14x1mm**
**Feeding Cell Suspension into Sphere Ring**

- Connect Bag to Syringe. Make sure that connected properly.
- Filter suspension with strainer and feed into Syringe.
- Release clip to take in.
- Use 50ml Syringe and inflate Bag with 60ml of air. To avoid bubbles, stand bag Upright.
- Culture on Orbital Shaker in incubator. Set at 45-55rpm.

**Collect Spheroids**

- Take into Syringe.
- Feed into a dish.
- Slowly rotate to gather at center.
- Collect

**Feed into Net Mold and Culture**

1: **Wet Feed Base**

- Wet Feeding Base with Medium. (This breaks surface tension and makes feeding spheroids easy. Remove Top net of Net Mold and place Net Mold on atop.

2: **Feed Spheroids into Net Mold**

- Wet bottom net by applying PBS by pipette. Slowly fill in spheroids into the Cavity. Make sure that 120% volume is fed, so that Spheroids are touching each other and not moving around.

3: **Set Top Net.**

- Place Top Net & Lock Plate. Make sure Teeth of lock Plate is pointing down.
- Put Stopper Pins & Holding Tubes. DO NOT FORGET TO REMOVE FEILLING BASE!

4: **Culture**

- Place filled Net Mold in 6Well Plate Or other container.
- Fill with Medium.
- Set at 30-40rpm (Swing Type)
- Change medium as necessary.
Net Mold “NM14-1”

<table>
<thead>
<tr>
<th>Model Name</th>
<th>NM14-1</th>
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<tbody>
<tr>
<td>Dimension</td>
<td>32x32x12mm</td>
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</table>
| Material       | Base: Polycarbonate  
                      Net: Stainless Steel  
                      Bolt: Stainless Steel  
                      Tube: Silicone  
                      Stopper: Silicone/Stainless Steel |
| Spare Parts    | Lock Plate X 5  
                      Top Net X 5  
                      Side Net X5  
                      Stopper x 5  
                      Holding Tube x5 |

**Top/Bottom Net** (same as top)

**Lock Plate**  
Teeth Upward  
Base  
Filling Base

**Side Net**  
X10 Pieces  

| t=0.1mm  
| Total 1.0mm |

**Holding Tube**
**Stopper**
**Lock Plate**  
Teeth Downward

*Teeth are bent to engraved Side.

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1: Remove Top Net

Remove Holding Tube & Stopper Pins.  
Pick up Top Net & Lock Plate.  
Wet Filling Base with PBS or Medium.  
Place Net Mold onto Filling Base.

2: Fill into Cavity

Wet Feeding Base with Medium.  
(This breaks surface tension and makes feeding spheroids easy.)

3: Place Top Net

Wet bottom net by applying PBS by pipette.  
Slowly fill in spheroids into the Cavity.  
Make sure that 120% volume is fed, so that  
Spheroids are touching each other and not  
moving around.

4: Place Top Net

Place Top Net & Lock Plate.  
Make sure Teeth of lock Plate is  
pointing down.  
Put Stopper Pins & Holding Tubes.  
DO NOT FORGET TO REMOVE FEILLING  
BASE!

5: Maturation

Place filled Net Mold in 6Well Plate  
Or other container.  
Fill with Medium.  
Set at 30-40rpm (Swing Type)  
Change medium as necessary.

6: Remove Nets

Remove Top Net & Lock plate.  
Slide out Side Nets.  
Tissue may enwrap Bottom Net.  
Leave on the Bottom Net to  
maintain shape.