

# FCeM<sup>®</sup>-series Preparation Kit500

## INSTRUCTIONS FOR USE

### DESCRIPTION

The FCeM<sup>®</sup> -series Preparation Kit500 consists of all the components needed to transform any 2D medium into a 3D medium for suspension culture of cancer cells, ES cells and iPS cells. The FP001 polymer, included in the FCeM<sup>®</sup> -series Preparation Kit500, can be added to any 2D medium to culture any cells in suspension. The viscosity of the 3D cell culture medium with FP001 polymer is same as that of water. The FP001 polymer prevents sedimentation, and uniformly disperses spheroids without agitation.

### COMPONENTS



- (1) Exclusive use conical tube (225 mL, 1 piece, sterile)
- (2) Specially-shaped adapter cap (1 piece, autoclavable<sup>1</sup>, sterile)
- (3) Syringe (20 mL, 1 piece, sterile)
- (4) Plastic flexible needle (1 piece, sterile)
- (5) FP001 Solution<sup>2</sup> (17 mL × 1 bottle, sterile)
- (6) Cell strainer (100 μm, 1 piece)

<sup>1</sup> at 121 °C for 20 min    <sup>2</sup> Storage at 2 - 30 °C, DO NOT FREEZE

### MATERIALS TO BE SUPPLIED BY THE USER

- Aseptic work area (clean bench, biosafety cabinet)
- Pipettor and pipettes (25 - 50 mL)
- Basal medium in 500 mL bottle for 3D cell culture

### MEDIUM TYPES AND MIXING RATIOS

The following example is for preparing 500 mL of the 3D medium.

Basal Medium	Volume (mL)		
	FP001 Solution	Basal Medium	Medium (Final)
DMEM-LG, DMEM-HG, EMEM, DMEM-Ham's F12, Essential™ 8, mTeSR-1, StemFit	10	200	500
RPMI1640	13	200	500

**WARNING!** A 3D dispersion mechanism is caused by the interaction between FP001 and components of the medium; therefore, in some cases 3D dispersion effects will not be observed depending on the composition of the basal medium selected. The 3D dispersion effects is lost by freezing of the 3D medium.

### CONTACT US

If you have any questions related to these instructions, encounter problems (i.e., errors incurred during 3D media preparation), or need help, please contact us by email, phone, or fax.

### WARNING

- This product ("Product") is designed for research and development use only – Do not use it for other purposes.
- Wear appropriate protective eyewear, clothing, and gloves when handling the Product.
- Avoid skin and eye contact, inhalation of vapors, or ingestion.
- No warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non-infringement, except as provided for herein.
- Nissan Chemical Industries, Ltd. shall not be liable for any damages as the result of (i) misuse, fault or negligence of or by users or purchasers of the Product, (ii) use of the Product in a manner for which it was not designed, or (iii) improper storage and handling of the Product.

### PREPARATION OF THE 3D MEDIUM

- ① Warm basal medium to room temperature ~ 37 °C.
- ② Dispense 200 mL of basal medium without serum into the 225 mL conical tube (1).
- ③ Replace the conical tube's cap (1) (light green) with the adaptor cap (2).
- ④ Attach the plastic flexible needle (4) to the 20 mL syringe (3).
- ⑤ Aspirate the prescribed amount<sup>†</sup> of FP001 solution (5) into the 20 mL syringe (4), and remove the plastic flexible needle from the FP001 sol. filled syringe (‡ See the TABLE).
- ⑥ Connect the FP001 sol. filled syringe (5) to the adaptor cap on the conical tube (3), and build up the medium manufacturing unit.
- ⑦ **FP001 SOLUTION MUST BE INJECTED RAPIDLY (WITHIN 5 SEC) into the basal medium** in the conical tube (6) while tightly holding the medium manufacturing unit.
- ⑧ Remove the syringe and the adaptor cap from the conical tube and screw on the conical tube's cap (1) (light green) to the conical tube. Then mix the solution gently.
- ⑨ Replace the cap of 500 mL basal medium bottle (containing 300mL of the basal medium) with the cell strainer (6).
- ⑩ Transfer the whole volume of the solution (8) to the 500 mL bottle (9) using a pipette (ex. Capacity 25 - 50 mL). When adding the solution, use the pipette by pushing vertically against the cell strainer at a speed mode of F (fast).
- ⑪ Remove the cell strainer (6), and screw the basal medium bottle's cap to the bottle. Then mix it gently.
- ⑫ After overnight incubation at 4 °C\*\*, add any further additives required for your cell line (e.g., serum, antibiotics, growth factors) to the 3D medium and initiate the 3D cell culture.

\*\* DO NOT FREEZE, store at 2 - 8 °C

