

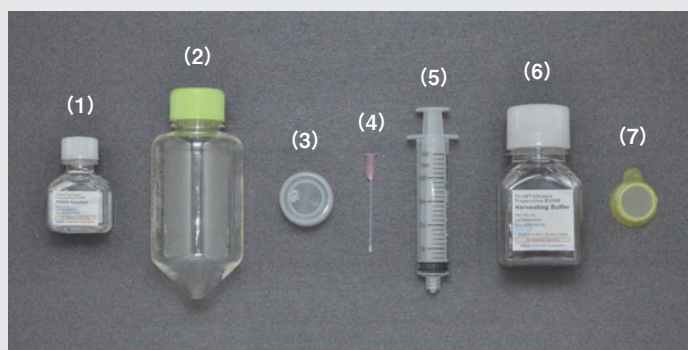
# FCeM<sup>®</sup> Advance Preparation Kit500

## INSTRUCTIONS FOR USE

### DESCRIPTION

The FCeM<sup>®</sup> Advance Preparation Kit500 is a convenient easy-to-manufacture kit optimized to produce a 3-D cell culture medium. The prepared medium using this kit can be used as a dispersion culture medium for adherent cells with low-attachment vessel. The Harvesting Buffer is used for recovering cells from 3-D cultured cell suspension.

### COMPONENTS



- (1) FP003 Solution<sup>†</sup> (15 mL x 1 bottle, sterile)
- (2) Conical tube (1 piece, autoclavable, sterile)
- (3) Adapter cap (1 piece, autoclavable, sterile)
- (4) Plastic flexible needle (1 piece, sterile)
- (5) 20 mL syringe (1 piece, sterile)
- (6) Harvesting Buffer (110 mL x 1 bottle, sterile)
- (7) 100 µm cell strainer

<sup>†</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 (50 mL)
- Basal medium

### MEDIUM TYPES AND MIXING RATIOS

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

Basal Medium	Volume (mL)		
	FP003 Solution	Medium (Preparation)	Medium (Final)
DMEM, EMEM, DMEM/F12 mTeSR <sup>™</sup> -1, TeSR <sup>™</sup> -E8 <sup>™</sup> Essential 8 <sup>™</sup> etc.	10	200	500
RPMI1640	13	200	500

**WARNING!** 3-D dispersion mechanism depends on the interaction between FP003 and components of the medium; therefore, in some cases 3-D dispersion effects may not be observed due to the composition of the basal medium selected.

### CONTACT US

If you have any questions related to these instructions, encounter problems (i.e., errors incurred during 3-D 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 Corporation 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 3-D MEDIUM

- ① Warm basal medium (without serum) to be used for 3-D culture to 37 °C.
- ② Dispense 200 mL of the warmed basal medium into the 225 mL conical tube (2).
- ③ Replace the conical tube's cap (2) with the adapter cap (3).
- ④ Attach the plastic flexible needle (4) to the 20 mL syringe (5).
- ⑤ Aspirate the FP003 solution<sup>†</sup> (1) into the 20 mL syringe (4), and remove the plastic flexible needle from the FP003 filled syringe. (‡ See the TABLE)
- ⑥ Connect the FP003 filled syringe (5) to the adaptor cap on the conical tube (3) to build up the medium preparing unit.
- ⑦ **INJECT THE FP003 SOLUTION AS QUICKLY AS POSSIBLE (WITHIN 5 SEC)** into the basal medium in the conical tube (5) while tightly holding the medium preparing unit.
- ⑧ Remove the syringe and the adaptor cap from the conical tube, and then screw the conical tube's cap (2) (light green) to the conical tube.
- ⑨ Replace the 500 mL basal medium bottle's cap with the cell strainer (6).
- ⑩ Transfer the whole volume of the solution (8) to the 500 mL bottle (9) by a pipette (ex. Capacity 50-100 mL). When adding the solution, use the pipette by pushing vertically against the cell strainer.
- ⑪ Remove the cell strainer (6), and screw the basal medium bottle's cap to the bottle. Then mix the solution gently.
- ⑫ After overnight incubation at 4 °C, add any further additives required for your cell line (e.g., antibiotics, growth factors) to the 3-D medium\* and initiate the 3-D cell culture.

\*DO NOT FREEZE THE 3-D MEDIUM; STORE AT 2-8°C

