

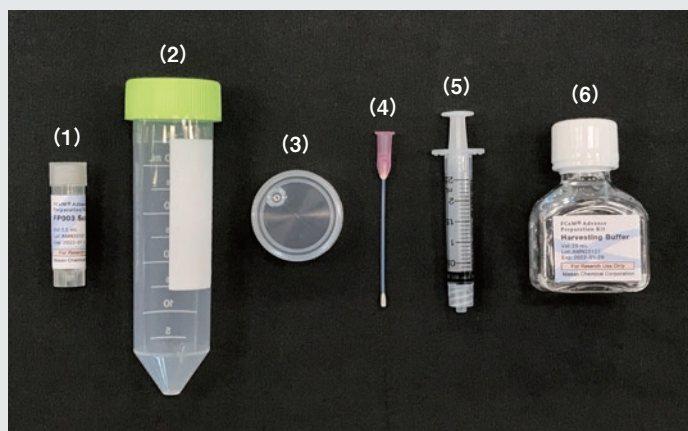
FCeM[®] Advance Preparation Kit

INSTRUCTIONS FOR USE

DESCRIPTION

The FCeM[®] Advance Preparation Kit 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 in low-attachment vessel without agitation. The Harvesting Buffer is used for recovering cells from 3-D cultured cell suspension.

COMPONENTS



- (1) FP003 Solution[†] (1.2 mL × 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) 2.5 mL syringe (1 piece, sterile)
- (6) Harvesting Buffer[†] (25 mL × 1 bottle, sterile)

† 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 or 50 mL)
- Basal medium for 3D culture

MEDIUM TYPES AND MIXING RATIOS

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

Medium for 3D culture	Volume (mL)	
	Basal Medium [‡]	FP003 Solution
DMEM, DMEM, EMEM, DMEM-Ham's F12, mTeSR™-E8™, Essential 8™, StemFit® AK02N etc.	49.2	0.8
RPMI1640	49.0	1.0

‡ To prepare 3D medium less than 50 mL, please decrease FP003 solution in the same dilution ratio according to basal medium volume. The recommended minimum volume of 3D medium is 30 mL.

WARNING! A 3-D dispersion mechanism is caused by the interaction between FP003 and components of the medium; therefore, in some cases 3-D dispersion effects may not be observed depending on the composition of the basal medium selected.

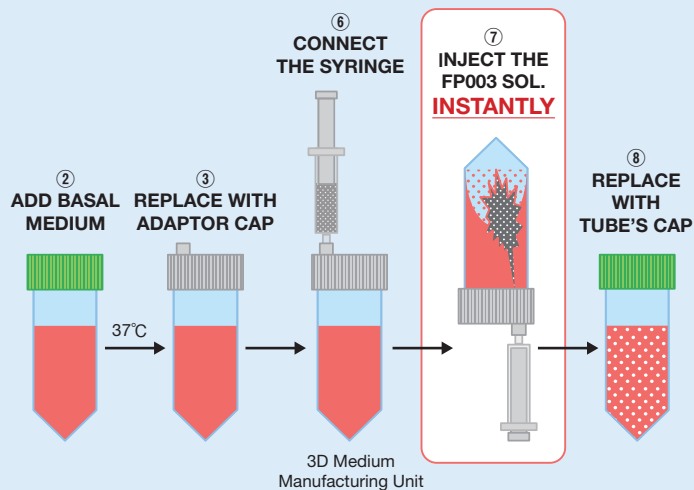
WARNING

- 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 using 3-D cell culture to 37 °C.
- ② Dispense basal medium for 3-D cell culture into the 50 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 2.5 mL syringe (5).
- ⑤ Aspirate the FP003 solution (1) into the 2.5 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 with the conical tube (3), and build up the medium manufacturing unit.
- ⑦ **INJECT THE FP003 SOLUTION AS QUICKLY AS POSSIBLE (WITHIN 0.5 SEC)** into the basal medium in the conical tube (5) while tightly holding the medium manufacturing unit.
- ⑧ Remove the syringe and the adaptor cap from the conical tube, and then screw on the conical tube's cap (2) (light green) to the conical tube. Then mix the solution gently.
- ⑨ After overnight incubation at 4 °C, add any further supplement 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



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.