3-Dimensional Cell Culture Medium

FCeM®-series Preparation Kit

INSTRUCTIONS FOR USE

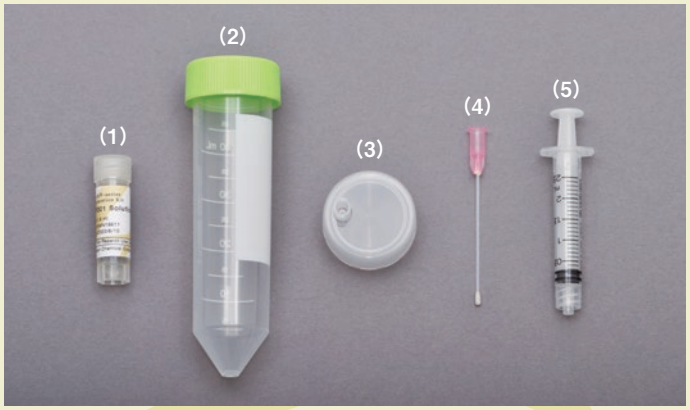
WARNING

- 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 ofthe Product, (ii) use of the Product in a manner for which it was not designed, or (iii) improper storage and handling of the Product.

DESCRIPTION

The FCeM®-series Preparation Kit 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®-series Preparation Kit, 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) FP001 Solution† (1.6 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)
† Storage 2-30 °C, DO NOT freeze.

MATERIALS TO BE SUPPLIED BY THE USER

- Aseptic work area (clean bench, biosafety cabinet)
- Pipettor and pipettes (25 mL or 50 mL)
- Basal medium

MEDIUM TYPES AND MIXING RATIOS

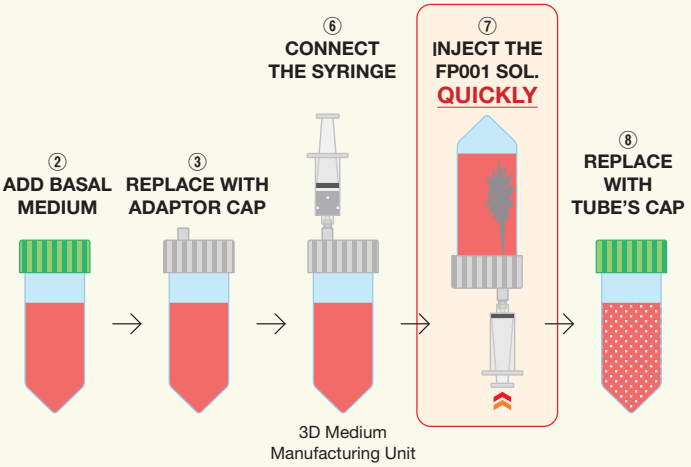
The following example is for preparing 50 mL of the 3-D medium. Adjust accordingly if preparing other volumes. The recommended medium volume is 30-50 mL.

Basal Medium	Medium (mL)	FP001 sol. (mL)
DMEM-LG, DMEM-HG, DMEM-Ham's F12, EMEM, Ham's F12, McCoy's 5A, etc.	49	1.0
RPMI1640	48.7	1.3
mTeSR™1, TeSR™ Family, Essential 8™, etc	48.5	1.5

PREPARATION OF THE 3D MEDIUM

1. Warm basal medium to be used for 3-D culture to 37 °C.
2. Dispense the prescribed amount‡ of basal medium into the 50 mL conical tube (2). (‡ See the TABLE)
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 FP001 solution‡ (1) into the 2.5 mL syringe ④, and remove the plastic flexible needle from the FP001 filled syringe .
6. Connect the FP001 filled syringe ⑤ to the adaptor cap with the conical tube ③, and build up the medium preparing unit.
7. **INJECT THE FP001 SOLUTION AS QUICKLY AS POSSIBLE (WITHIN 0.5 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. 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**



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! 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.

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