

SARS-CoV-2 Spike V367F mutant Cell Line

Culture Instruction Manual

Materials & Reagents Required

Dulbecco's Modified Eagle's Medium - high glucose (Ref: D6429 Sigma-Aldrich)
MEM Non-essential Amino Acid Solution (100x) (M7145 Sigma-Aldrich).
Fetal Bovine Serum (FBS)
Hygromycin
DPBS (Ca²⁺ & Mg²⁺ free)
Incubator, 37 °C/5% CO₂.
Tissue culture vessels
Water Bath, 37 °C
15 mL tubes.
Centrifuge
Pipette
Ice

Complete Growth Medium

Dulbecco's Modified Eagle's Medium - high glucose (Ref: D6429 Sigma-Aldrich)
MEM Non-essential Amino Acid Solution (100x) (M7145 Sigma-Aldrich).
Fetal Bovine Serum (10%)
Hygromycin (80 µg/ml)

Any changes in the experimental conditions may have negative effects on cell survival and may yield abnormal cell experiment. For more information and for a complete list of Innoprot's reagents and products contact our customer service.

General Considerations: The protocols included in this manual are intended to serve as a guide only, and optimization of culture protocols is encouraged to ensure success.

| 1.0 IMMEDIATELY UPON DELIVERY | |
|-------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1.1 | Remove vial from shipping container to check that it is still frozen. |
| 1.2 | Transfer frozen vial to liquid nitrogen until you are ready to thaw and begin cell culture. |
| 2.0 THAWING CELLS | |
| 2.1 | Prepare necessary " Thawing medium " and warm prior to plating cells: <ul style="list-style-type: none"> • DMEM High Glucose (D6429 from Sigma-Aldrich) • 10% FBS |
| 2.2 | Thaw cells rapidly. Place the vial in a 37°C waterbath, hold and rotate the vial gently until the contents are completely thawed. Do not allow sample to warm to 37°C. Cryovials should be cool to the touch when removed from bath. Passive thaw is not recommended. |
| 2.3 | Remove the vial from the waterbath immediately, wipe it dry, and transfer it to a sterile field. |
| 2.4 | Rinse the vial with 70% ethanol, and then wipe to remove excess. Remove the cap, being careful not to touch the interior threads with fingers. Using 1 ml eppendorf pipette gently resuspend the contents of the vial. |
| 2.5 | Add warm media to a 15 mL tube until the 8 ml demarcation. |
| 2.6 | Immediately transfer contents of vial to the 15 mL tube. Gently invert the tube to distribute contents. |
| 2.7 | Centrifuge at 1.500 r.p.m. for 5 minutes. Remove supernatant and resuspend cell pellet in warm medium |
| 2.8 | Count the cells and dispense the contents of the tube into a T-25 flask. |
| 2.9 | Place the flask to the incubator |
| 2.10 | For best result, do not disturb the culture for 24 hours after the culture has been initiated. Change the growth medium (including Hygromycin 80 µg/ml) the next day to remove unattached cells, then every other day thereafter. |

| 3.0 MAINTENANCE OF THE CULTURE | |
|---------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 3.1 | Change the medium to fresh supplemented medium the next morning after establishing a culture from cryopreserved cells. For subsequent subcultures, change medium 48 hours after establishing the subculture. |
| 3.2 | Once the culture reaches 50% confluence, change medium every day until the culture is approximately 80% confluent. |
| 3.3 | Subculture the cells when they are over 90% confluent. |
| 4.0 SUBCULTURING | |
| 4.1 | Warm medium, trypsin/EDTA solution and DPBS to room temperature. We do not recommend warming the reagents and medium at 37 °C waterbath prior to use. |
| 4.2 | Rinse the cells with DPBS. |
| 4.3 | Add 1 ml of trypsin/EDTA solution into flask (in the case of T-25 flask); gently rock the flask to make sure cells are covered by trypsin/EDTA solution; incubate the flask at 37°C incubator for 1 to 2 minutes or until cells are completely rounded up (monitored with inverted microscope). During incubation, prepare a 15 ml conical centrifuge tube with 5 ml of FBS; transfer trypsin/EDTA solution from the flask to the 15 ml centrifuge tube (a few percent of cells may detached); at the end of trypsinisation, with one hand hold one side of flask and the other hand gently tap the other side of the flask to detach cells from attachment; check the flask under inverted microscope to make sure all cells are detached, add 5 ml of complete medium to the flask and transfer detached cells to the 15 ml centrifuge tube; add another 5 ml of complete medium to harvest the residue cells and transfer it to the 5 ml centrifuge tube. Examine the flask under inverted microscope to make sure the cell harvesting is successful by looking at the number of cells left behind. There should be less than 5%. |
| 4.4 | Centrifuge the 15 ml centrifuge tube (harvested cell suspension) at 1200 rpm (Beckman Coulter Allegra 6R centrifuge or similar) for 5 min; re-suspend cells in growth medium. |
| 4.5 | Count cells and plate cells in a new flask with cell density as recommended. |