GREEN FLUORESCENT RKO Cell Line

Culture Instruction Manual

Materials & Reagents Required

Dulbecco's Modified Eagle's Medium - high glucose (Ref: D6429 Sigma-Aldrich)
Fetal Bovine Serum (FBS)
MEM Non-Essential Amino Acid Solution (100×) (M7145 Sigma-Aldrich)
G418
DPBS (Ca^{2+} & Mg^{2+} free)
Incubator, 37 °C/5% CO_2.
Tissue culture vessels
Water Bath, 37 °C
15 mL tubes.
Centrifuge
Pipette
Ice

Complete Growth Medium

DMEM (Ref. D6429 Sigma-Aldrich)
Fetal Bovine Serum (10%)
MEM Non-Essential Amino Acid Solution (100×) (M7145 Sigma-Aldrich)
G418 (250µg/ml)

The cell culture protocols described in this manual include the *in vitro* culture of Green Fluorescent RKO Cell Line. 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.

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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: • DMEM (Ref: D6429 Sigma-Aldrich) • 10% FBS
2.2	**Thaw cells rapidly**. Place the vial in a 37ºC waterbath, hold and rotate the vial gently until the contents are completely thawed. Do not allow sample to warm to 37ºC. 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 16 hours after the culture has been initiated. Change the growth medium (including G418 250µg/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 ºC waterbath prior to use.
4.2	Rinse the cells with DPBS.
4.3	Add 2 ml of trypsin/EDTA solution into flask (in the case of T-75 flask); gently rock the flask to make sure cells are covered by trypsin/EDTA solution; incubate the flask at 37ºC incubator for 2 to 5 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 thawing medium; transfer trypsin/EDTA solution from the flask to the 15 ml centrifuge tube. Check the flask under inverted microscope to make sure all cells are detached, add 5 ml of thawing medium to the flask and transfer detached cells to the 15 ml centrifuge. 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 1000 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. Subculture Ratio: 1:3 to 1:12 Medium Renewal: Every 2 to 3 days.