Jurkat Linterna

Culture Instruction Manual

Materials & Reagents Required

RPMI 1640 Medium (Ref: 61870010 Gibco)
Fetal Bovine Serum (FBS Heat inactivated)
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

RPMI 1640 Medium (Ref: 61870010 Gibco)
Fetal Bovine Serum (10%)
G418 (150µg/ml)

The cell culture protocols described in this manual include the *in vitro* culture of. 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: • RPMI 1640 Medium (Ref: 61870010 Gibco) • 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-75 flask for non-adherent cells.
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 G418 150µg/ml) the next day to remove unattached cells, then every other day thereafter.

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3.0 MAINTENANCE OF THE CULTURE AND SUBCULTURING	
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	When cells are 6-8x10^5 cells/ml, split them 1:4 with fresh media. Add appropriate aliquots of the cell suspension to new culture vessels (T25 = 10 ml; T75 = 50 ml; T150 = 100 ml maximum volume).
3.3	Grow cells to no more than 8 x 10^5 cells/ml. Disperse clumps gently for counting.

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