P30701 - APP Processing Assay
Division-Arrested APP-tGFP MDCK Cells

Culture Instruction Manual & Assay Protocol

Materials & Reagents Required

DMEM high glucose
MEM NEAA
Fetal Bovine Serum (FBS)
DPBS (Ca^{2+} & Mg^{2+} free)
Formaldehyde Solution
DAPI Solution
Incubator, 37 °C/5% CO_2.
Tissue culture vessels
Water Bath, 37 °C
15 mL tubes.
Centrifuge
Pipette
Ice

*The cell culture protocols described in this manual include the *in vitro* culture of APP-tGFP MDCK cells and APP Processing Assay. 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.

Division-Arrested Cells from Innoprot are prepared to use immediately upon thawing. Each batch of Division-Arrested Cells has been highly validated showing the same response to the reference compounds than stable cell lines.

Division-Arrested Cells are not recommended to be subcultured since these cells have been treated with Mitomycin-C (MMC) to generate mitotically inactive feeder cells.

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 & ASSAY PREPARATION	
2.1	Prepare necessary "***Thawing medium***" and warm prior to plating cells: • DMEM high glucose • 10% FBS • MEM NEAA • Gentamicin
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	Immediately transfer contents of vial to a 15 mL tube
2.6	Drop wise add warm media until the 8 ml demarcation. 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 the assay culture vessels. Approximately a seeding density of 45,000 cells/cm^2 is recommended (15.000 cells/well in 96-well plates).
2.9	Cover the plate and gently rock the vessel to distribute the cells evenly. Loosen caps if necessary to permit gas exchange.
2.10	Incubate in a humidified 37ºC / 5% CO_2 incubator overnight.

3.0 ASSAY PROCEDURE	
3.1	Prepare "***Assay medium***" and warm prior to treat cells: • DMEM high glucose • 1% FBS
3.2	Remove "thawing medium" and replenish with "Assay Medium"
3.3	Add reference compounds or test compounds
3.4	Incubate cells in a humidified 37ºC / 5% CO^2 for 3 days (72 hours).
3.5	***Fix Cells - Highly Recommended but not necessary*** 1.- Remove assay medium 2.-Fix cells adding 3.7 % formaldehyde during 20 minutes at room temperature. 3.- Remove formaldehyde and wash twice with DPBS
3.6	***Stain Nucleus with DAPI - Highly Recommended but not necessary*** 1.- Stain nucleus with DAPI during 5 minutes at room temperature 2.- Remove DAPI solution and wash twice with DPBS 3.- Depending on the analysis device it could be recommended to maintain the cells in DPBS.
4.0 DATA ANALYSIS	
4.1	APP spots will be detected by fluorescence
4.2	Quantify vesicle number using image analysis algorithms
4.3	Quantify cell number using image analysis algorithms
4.4	Calculate retained vesicle number per cell.