AR 42J cells

- DMEM 10%FCS- Penicilin-Streptomycin-Glutamine
- thaw frozen vial in 10 ml media
- spin the cells at 2000 rpm for 5 min
- resuspend the cells in 10 ml media and plate in a 10cm dish
- the cells might take about 24h to adhere to the plate
- once cells are attached to the plate, change media
- 2 days after plating, even if the cells did not grow much, it's best to split them (1:1 or 1: 2 if they grew) in order to remove cell debris. You will see some cell debris at this point. They might look like contamination but are not.
    - wash the cells with PBS
    - add 3 ml trypsin and tap the plate up to the point when the cells start to detach. AR 42J cells do not like to be trypsinized for a long time
    - neutralize the trypsin with 7 ml media
    - resuspend the cells by pipetting up and down
    - centrifuge the cells (this helps to remove cell debris)
    - resuspend cell pellet by pipetting up and down
    - plate into 1 or 2 plates
- change media every day
- repeat previous step (trypsiniz-spin-plate in fresh plate) up to when you get a confluent plate. This usually takes about a week.
- After I get a confluent plate, I split cells 1:4 every 2 days. At this point, I simply trypsiniz the cells, neutralize tripsin with media and split in 4 plates (no centrifugation step is required when the cells are happily growing)
- Again, it's important to trypsiniz the cells for the minimum amount of time required to detached them from the plate. Also, to get the cells to a single cell suspension, you need to pipette up and down a few times. Indeed, AR 42J cells have the tendency to grow in clusters looking like raspberries.

- It’s important to change media every day. One can get a lot of cells per plate and it’s important, for ER stress studies that the cells are well fed. Nutrient deprivation can lead to ER stress (PERK and IRE activation).
- Typically, I plate the cells on Day 1, change media on Day 2 and do the experiment on Day 3. In the morning of day 3, I place the cells in fresh media for 5h before starting to treat the cells. In these conditions, I have untreated controls that shows minimal basal activation (Bertolotti, A., Zhang, Y., Hendershot, L.M., Harding, H.P., and Ron, D. Dynamic interaction of BiP and the ER stress transducers in the unfolded protein response. Nature Cell Biology 2: 326-332 (2000).

Good luck with your experiment

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