

10mm dish cell scraping protocol

Materials (2x 10mm dish)

warm 5 ml EDTA DPBS (such as versene) for each plate

two 15 ml falcon tubes

(tube 1: saved media, tube2: scraped cells)

Objective

-scrape two 70-90% confluent 10 cm dishes of Kv2.1 TREX cells incubated with 1 ug/ml tetracycline for 24-48 hours (1 dish for every 2 tubes to be incubated with beads)

-Perform the same procedure for 2x 10cm dishes of Empty CHO cells.

-to re-suspend a dense amount of cells to plate with beads 5 and 180 K solution after appropriate incubation time

Procedure (for 2x 10cm dishes of a given cell type)

-remove cell media from dishes and save in 15 ml tube

-rinse cell plate with 5mL of EDTA DPBS, aspirate majority of solution leaving a thin layer of EDTA DPBS on cells

-scrape entire plate surface area of cells

-place all of scraped cells in a separate 15mL falcon tube (2)

-rinse dishes with 5mL of their **original** cell media from tube (1), and add to cell mix

-centrifuge tubes at 500g for 5 minutes; cells will pellet at bottom

-remove **supernatant** from cell mix, careful not to remove cell pellet formed on bottom.

-re-suspend cells in 4 ml of the **supernatant** media (tube 2) you've just spun down, triturate 10X to break up cell clumps, and transfer to 2x 2mL tubes

Questions for Jon... NOTE: Should we have all the cells in a high density 1x 1.7ml tube, or re-suspend cells then transfer equally to 2x 1ml into 1.7ml tubes?

Clearly state in protocol how much cells should be in final tube and then how much should be transferred? Mix empty with kv 2.1, and perform experiment completely blind? Or set up individual tubes with isolated cell type? Logistically, the double blind test seems difficult due to the fact its a lot of counting for one person given the time intervals is another pressure.

add beads ASAP, keep incubating at 37 degree water bath