

Dharmacon siRNA transfection reagent transfected in K562 cell protocol

- 1.) Dharmacon siRNA on target plus Smart pool (1-4) dissolved in 1X siRNA Buffer (5X siRNA Buffer Dharmacon Thermo Scientific, cat.no. #B-002000-UB-100).
- 2.) Adjust the concentration of siRNA in such way that its final concentration is 50nM in a total final volume of 2ml.
- 3.) Add 100 ul of 1X siRNA buffer in the original siRNA vials(ordered from Dharmacon Thermo Scientific as 2nmol)
- 4.) Then it becomes 20UM stock.
- 5.) Keep dissolving the siRNA for 30 minutes at room temperature with gentle shaking, then take quick spin and collect the tubes, they can be stored at -20^0C freezer for longer time for future use.
- 6.) From this 20UM stock, take 18.75 ul of diluted siRNA in 1XsiRNA Buffer and add 981.25 ul of 11X siRNA Buffer to make 1ml of 375nM siRNA substock.
- 7.) From this 375nM substock,take 266ul of siRNA in a new and clean 1.5ml Eppendorf microcentrifuge tube, then add 4ul of Dharmafect transfection reagent1(cat.no.#T-2001-03 ,Thermo Scientific).
- 8.) Mix and keep the microcentrifuge tube inside of the cell and tissue culture room Hood.
- 9.) Add the siRNA and Damarfect1 reagent mix to one well of 6 well cell tissue culture plate.
- 10.) To this mix on the top, add K562 cells such that we have about 1×10^6 cells in 500ul of OPTI MEM I 1X (GIBCO, Invitrogen cat.no.31985).
- 11.) Incubate the plate for 30mins at Room Temperature in the Hood.
- 12.) Add 1234 ul of ~~OPTI~~ MEM I medium to the well of the 6 well plates and get the final total volume of 2ml.
- 13.) In the cell medium, the final siRNA concentration should be 50nM.
- 14.) incubate the K562 cell siRNA tranfection medium at 37^0C ,5% CO2 Cell and Tissue Incubator for siRNA tranfection

experiment for 3 or 4 Days to see efficient knock down assayed by Western Blot and or QPCR.

* All the steps above should be performed strictly in the Cell and Tissue Culture Hood.