NF-kB reporter assay

1. plate cells in a 6-well plate.
   (100% confluent 10cm plate =14wells of 70% confluent 6well plate if you grow it for o/n)
2. mix DNA (including GFP 20ng, renilla 5ng, firefly 10ng up to 3ug – use smaller total volume)
3. transfect cells with 3ug DNA and 7ul of lipofectamine per well

① dilute 3ug of DNA into 250ul DMEM

② dilute 7ul of lipofectamine into 250ul DMEM incubate for 5’ @ r/t

③mix DNA and lipofectamine , incubate for 20’ @ r/t

④add 500ul of mixture per well

⑤ change medium after 4-6hrs (optional)

4. harvest cells
   - medium suction -> wash with PBS -> add 1ml PBS and blow off cells -> collect cells in e-tube ->spin @ 1200rpm for 1’ -> pour off supernatant-> add 500ul PBS-> split to two tubes ->spin down cells-> pour of supernatant -> freeze at -20C

5-1. Dual luciferase assay

①add 100ul of 1x PLB(passive lysis buffer)/tube

② vortex it gently, leave for 10’

③ spin at max speed for 1’ (supernatant- cytosol, pellet – nucleus)

④ use 20ul for each assay

⑤ make Stop& Glo reagent (prepare it fresh)

⑥ check luciferase activity

- turn power on -> others-> oper.func->reagent-> prime *2
- measure -> protocols-> 5-> enter->yes
- when ended-> exit-> others-> oper.func-> reagent-> prime *2 -> prime with dH2O *2 -> prime with air*2 -> turn off

5-2 Quantitative Western

① cell lysis in 50ul of 1xNET, 0.5% Triton X-100, 1mM PMSF at r/t

② spin at max speed for 2’ , take supernatant

③ add sample buffer and boil at 95-98C for 2’

④ run at 9% SDS-PAGE for running, 5% SDS-PAGE for stacking gel

⑤ transfer to nitrocellulose memb

⑥ block with 5% milk

⑦ primary Ab (1:500) for 1hr

⑧ secondary Ab (1:1000) for 1hr

6. Alkaline Phosphatase reaction
- add 66ul of NBT stock + 66ul of BCIP to 10ml AP buffer
- develop at r/t for at least 10min
- to stop, wash the memb under tap water

7. Quantitate the amount of protein by using Image Quant software