

cDNA prep for qPCR using SuperPrep® II Cell Lysis & RT Kit for qPCR

(Toyobo SCQ-401, https://www.toyobo-global.com/seihin/xr/lifescience/products/cdna_004.html)

Day 0

Plate cells into 96 well @~ 8×10^4 /100 μ l/well

Day 1

↓ \pm Drug treatment

Day 2

↓ wash well w/ 100 μ l PBS

↓ lyse cells w/ 10 μ l* lysis buffer (10 μ l buffer + 0.166 μ l RNase inhibitor + 0.05 μ l gDNA remover from Toyobo super prep II cell lysis kit (SCQ-401), 5min, shaking using IKA MS 3 basic mixer @600/min

(x50= 500 μ l buffer, 8.3 μ l RNase inhibitor, 2.5 μ l gDNA remover)

*can be done w/ 20 μ l

↓

Take 4 μ l

↓ +2 μ l 5xRT mix (above kit), +4 μ l DW

(x50= 100 μ l 5xRT mix, 200 μ l DW)

37°C, 15min; 50°C, 5min; 95°C, 5min

↓ +140 μ l DW \rightarrow -20°C

Use 5 μ l from above for qPCR with Toyobo QPX-201, 10 μ l reaction, on StepOne

(x50= 250 μ l 2xMaster mix, 1 μ l** 100 μ M primer each, final @0.2 μ M)

Human GAPDH primer set

511_GAPDHf AGCCACATCGCTCAGACAC

512_GAPDhr GCCCAATACGACCAAATCC

**Can be done w/ 1.5 μ l (@0.3 μ M)