Chimeric Transcription Factor Assay Protocol

Material

Plasmids used. The schematic maps are shown above.
pCAG- relA/NLS/traR-polyA
$(trabox)_7$-CMVmin-AtIPT4-IVS-IRES-LOG(pIRESneo3)

Enzymes to linearize the plasmids for electroporation
pCAG- relA/NLS/traR-polyA was cut by *HindIII.*
$(trabox)_7$-CMVmin-AtIPT4-IVS-IRES-LOG(pIRESneo3) was cut by *PvuI.*

Medium
DMEM 10% FBS
DMEM 10% FBS G418 (400 μg/mL)

The primers for quantitative RT-PCR
・*atIPT4*
Forward: 5'- gtgcaacgacaaaatggtgg-3'
Reverse Sequence: 5'-gctaaccagcactagaagtcc -3'

・*log1*
Forward: 5'-ggactgatctctcaggctgtg-3'
Reverse: 5'-cgactacgtatagacgatggc-3'

Assay protocol
1, The EA.hy 926 cells were cultured to about $1.0 * 10^7$ cells/dish (the dish size is 10 cm in diameter) and used for electroporation.
2, C8 was added to the dish at final concentration of 0, 20, or 40 μM and incubated further for 24 hours.
3, After harvesting the cells, total RNAs were purified according to the ordinary AGPC (Acid guanidinium thiocyanate-phenol-chloroform) method, and cDNAs were obtained by reverse transcription.
4, qPCR was performed using the above cDNA.

[note]

AGPC methods

①Vortex the collected cells in the tube for 10 to 15 sec.

②Add 1/10 volume of Na acetate.

③Add 1.4-times volume of Phenol/Chloroform/Isoamyl alcohol.

④Vortex every 10 minutes.

⑤Centrifuge at 15000 rpm, 10 min, 4 ° C.

⑥Recover the aqueous layer and mix with 400 μL isopropanol.

⑦Leave at -20 ° C for 1 hour.

⑧Centrifuge at 15000 rpm, 10 min, 4 ° C and remove supernatant.

⑨Add 400 μL of 75% ethanol, centrifuge at 15000 rpm, 10 min, 4 ° C and remove supernatant.

⑩Dry the pellet and dissolve with 10 μL of pH = 7.4 TE buffer

Reverse transcription

Total RNA	8 μL
Oligo (dT)[10 pmol/ μL]	1 μL
5* RT buffer	4 μL
10mM dNTP	2 μL
ReverTra Ace	1 μL
RNase inhibitor	1 μL
dH2O	3 μL
total	20 μL

After mixing, samples were heated at 42° C. for 20 min, 99 ° C. for 5 min, 4 ° C. for 5 min, then stored at -20 ° C.

・qPCR

2*SYBR	10 μL
10 mM Primer F	0.4 μL
10 mM Primer R	0.4 μL
dH2O	6.2 μL
Sample	3 μL
total	20 μL

※SYBR：KAPA SYBR FAST qPCR kit