

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 μ g/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 μ M 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 μ L isopropanol.
- ⑦Leave at -20° C for 1 hour.
- ⑧Centrifuge at 15000 rpm, 10 min, 4° C and remove supernatant.
- ⑨Add 400 μ L of 75% ethanol, centrifuge at 15000 rpm, 10 min, 4° C and remove supernatant.
- ⑩Dry the pellet and dissolve with 10 μ L of pH = 7.4 TE buffer

Reverse transcription

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

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 μ L
10 mM Primer F	0.4 μ L
10 mM Primer R	0.4 μ L
dH2O	6.2 μ L
Sample	3 μ L
<hr/>	
total	20 μ L

※SYBR : KAPA SYBR FAST qPCR kit