

Half-Life Protocol

V3 NDJ 10.28.2004

Day 1 (Sat)

1. Plate 2.7×10^6 cells for p100.

Day 2 (Sun)

8 PM

2. Transfect p100 plates & p60 of CHOAA8(tTA) cells. Follow Transfection protocol. Cells grown in α MEM 10%FBS P/S G418
 - a. Use Lipofectamine transfection

	Condition	GFP-ataxin-1	Akt
1	Background Control (p100)	--	--
2	GFP Control (p100)	2ug	--
3	Akt-CA (p100)	2ug	2ug
4	Akt-NLS (p100)	2ug	2ug
E		2ug (A776)	--
F		2ug (E776)	--

Day 3 (Mon)

Evening

3. Wash with warm PBS
4. Add 2ml warm TE
5. Incubate 5 minute and bang on hand
6. Re-suspend in 8mls media
7. Spin down for 4minutes at 3-4 setting
8. Resuspend cells in 7ml media
9. Add 1ml to each p60 plate. Add 3ml media.

Day 4 (Tue)

Starting 8 AM

10. At 8AM add Dox (36 hours after transfection)
Add 1ug/ml. 1:1000 of 1mg/ml stock (4ul to each)
11. Collect time point by rinsing with PBS warm adding 500ul lysis buffer (see WB protocol.
12. Rock 4°C 15 minutes
13. Transfer to 1.5ml gel slick tube
14. Freeze -80°C
- 15.
16. Collect cells take time points
 - a. 0 hour (8AM)
 - b. 3 hour (11 AM)
 - c. 6 hour (2 PM)
 - d. 12 hour (8 PM)
 - e. 24 hour (8 AM Day 5)

Day 5 (Wed)

Starting at 8 AM

17. Collect final time point.
18. After all time points. Thaw extracts and follow WB protocol
 - a. 10x 21 gauge needle
 - b. 5x 25 gauge needle
 - c. Spin max 10 minute 4°C
 - d. Transfer supernatant to new gel slick tube
 - e. Freeze extracts
19. Bradford extracts.
20. Use file “mydocuments\templates\bradfordtempgfp.xls” to make calculations
21. Dilute in lysis buffer (75ug of protein in 350ul). Run each condition thrice (100ul each).

Day 6 (Thur)

ELISA

22. Wash black GFP plate 3x with PBS-T(0.5%) in plate washer
 - a. Get 96 well lid (can re-use)
 - b. Get GFP 96 well plate Reacti-bind plate (#15182 Pierce) out of deli cooler.
 - c. Turn on plate washer
 - d. Prime with water
 - > (Press) Yes (to home axis)
 - >Run
 - >Prime
 - >Prime Program 02(Nozzle should be attached to H₂O resevier)
 - >Enter
 - >Start
 - e. Prime with PBS-T
 - >Run
 - >Prime (Change nozzle to PBS-T resevier)
 - >Enter
 - >Start
 - f. Put plate in plate washer
 - h. Wash Plate
 - >Run
 - >Wash
 - >Program 5 Courtney 3x
 - >Enter
 - >Start
 - i. Switch nozzle to water for rest. (After 10-15 minutes, the washer will return to rest position automatically and soak the capillaries in H₂O.)
 - j. Dump liquid in sink
23. Add 100ul lysate to plate. Rock 1 hour. Covered. RT (room temperature)
24. Wash plate 3x PBS-T + 125ul PBS-T (It will pipette out lysates.)
 - a. Prime with PBS-T
 - >Yes (to home axis)
 - >Run

- >Prime
- >Prime Program 02(Change nozzle to PBS-T resevior)
- >Enter
- >Start
- b. Put plate in plate washer
- c. Wash Plate
 - >Run
 - >More
 - >Link
 - >Program CEB
 - >Enter
 - >Start
- d. Remove bubbles from wells with pipette if any

Switch nozzle to water for rest.

25. Read GFP

- a. >K4 Program
- b. >New plate icon
- c. >GFP icon
- d. >Wizard
- e. > Description
 - i. Bookpage
 - ii. Cell type
 - iii. Experiment
- f. > Reading Parameters >Options
 - i. High wells: set what is high (Akt-NLS/E776)
 - ii. High values 65000 (can lower marks)
 - iii. Starting sensitivity: 195
 - iv. >Okay >Okay >Okay
- g. >Read

24. Dump out PBS-T

- 25. Add 100ul PN1168 (1:1000 in PBS) Rock RT. 1 hr.
 - 26. Wash plate 3x PBS-T (plate washer)as in 22.
 - 27. Add 100ul 2° antibody to each well. Rock RT 1 hr
- **Take out TMB to warm

10ml 2° Antibody	
9ml	PBS
1ml	10mg/ml BSA (special)
5ul	anti-rabbit
10ul	tween 20

- 28. Wash plates 3x PBS-T plate washer as in 22.
- 29. Add 100ul RT TMB- Slow (stored in fridge)

(monitor blue color \approx 5 to 10 minute)

30. Add 50ul 4N NH_2SO_4 to stop reaction. On Kerri's bench.
31. Transfer 125ul to new, clear bottom plate
33. Read plate
 - a. >PN1168 Icon
 - b. >Wizard
 - >Description. Follow 25e.
 - c. >Read
 - d. Export as txt file.
 - e. Email to self.
34. Turn off

Day 7

33. Crunch Numbers