

## FILTER TRAP ASSAY FOR AGGREGATES

### **Materials/Reagents**

- 1X PBS
- 100 mM PMSF
- 10% SDS
- 1X PBS + 1% SDS
- Blocking solution
  - 1X PBS
  - 5% skim milk

### **Procedure** (For HeLa Tet-OFF httQ143YFP Cell line)

1. Cells are grown on 10 cm plate and induced for the expression of aggregate prone protein.
2. Harvest cells after washing with 1x PBS.
3. Resuspend in 100  $\mu$ l of 1x PBS + 1mM PMSF.
4. Sonicate in sonic bath for 1min for cell lysis and DNA cutting.
5. Protein assay by Bradford method.
6. Take 200  $\mu$ g total protein (before taking, vortex well), adjust the final volume to 100  $\mu$ l with PBS and add 10  $\mu$ l of 10% SDS.
7. Add 900  $\mu$ l of PBS+ 1% SDS and stay at RT.
8. Cut a cellulose acetate membrane and pre-wet with 1xPBS + 1% SDS.
9. Set the dot blotting apparatus and apply all (1.1 ml) in a slot (before loading sample, vortex well).
10. Wash with 1 ml of 1xPBS + 1% SDS twice and transfer membrane into Blocking solution. Do Western blotting for aggregate detection.

### **Troubleshooting/Critical Parameters**

- GST-fused htt-ex1Q80 expressed *E. coli* lysate may be used as a control. In this case, 50 $\mu$ g of *E. coli* lysate is enough for detection.
- Instead of doing sonication (step4), you can lyse cells by freeze-thaw method, add 5 $\mu$ l of DNase, and incubate for 1-2 hour at 37°C.
- As aggregates are stacked in a membrane pore (probably), you do not need to worry about washing out the aggregate during Western blotting.

### **References**

- Scherzinger, E. *et al.*, *PNAS*, **96**, 4604-4609, 1999  
Wanker, E.E. *et al.*, *Methods Enzymol.* **309**, 375-386, 1999

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