TRAP2: Fos2A-iCreERT2

(DeNardo et al., 2019)

Reagents

* 4-hydroxytamoxifen 4-OHT, H6278, Sigma
* Absolute EtOH, E/0650DF/15, Fisher Chemical
* Corn oil, C8267-500ml, Sigma
  + KEEP OIL STERILE

1. Dissolve 4-OHT in absolute EtOH by shaking 15 min at 37°C till dissolve

Concentration: 20mg/ml = 10mg/0.5ml = 50mg/2.5ml or 25mg/1.25 ml

* + Always wrap in aluminium foil: always seal from light

1. Aliquot (300ul); store at -20oC: up to weeks (in real life several months)
2. 1 day before behavior test

* measure mouse weight; calculate 4-OHT i.p. volume

Critical pt.: always prepare in excess to avoid loss

1. Redissolve 4-OHT in absolute EtOH by shaking for 15 min at 37°C till completely dissolved with thermomixer
2. Add 2x volume of Corn oil to dilute by half

EtOH is evaporated by vacuum under centrifugation – 15 mins, 45°C

till fully evaporate

**Final concentration: 10mg/ml, must be used fresh**

1. Prepare i.p injection volume in tube;

Keep i.p volume on 37 thermal block to avoid 4-OHT precipitation at R.T.

1. Bring mice from SPF (>5 days in advance), and in experimental room 30 min in advance
2. Behaviour Test
3. Immediate i.p injection of 50 mg kg–1=0.05mg g-1 4-OHT

e.g. for 30g mouse, (0.05x30)/10 = 0.15ml = 150ul

Crtiical pt.- 4-OHT i.p amount must be very precise; to minimize excess usage – use insulin syringes (BD Microfine+ 0.5ml 324825)

|  |  |
| --- | --- |
| Mouse weight (g) | 4-OHT solution volume (µl) |
| 16 | 80 |
| 17 | 85 |
| 18 | 90 |
| 19 | 95 |
| 20 | 100 |
| 21 | 105 |
| 22 | 110 |
| 23 | 115 |
| 24 | 120 |
| 25 | 125 |
| 26 | 130 |
| 27 | 135 |
| 28 | 140 |
| 29 | 145 |
| 30 | 150 |
| 31 | 155 |
| 32 | 160 |
| 33 | 165 |
| 34 | 170 |
| 35 | 175 |