

## Experimental Autoimmune Encephalomyelitis (EAE)

**EAE induction and treatment.** C57Bl/6 WT mice were immunized with 100µg MOG<sub>35-55</sub> peptide (MEVGWYRSPFSRVVHLYRNGK) (Biopolymers Laboratory of Harvard Medical School) emulsified in complete Freund's adjuvant (CFA) (Oil solution) {that is a solution (4mg/mL of mycobacteria (Difco) in incomplete Freund's adjuvant (IFA) (Difco))} subcutaneously (100µg per side flank = 100ul). On the day of immunization, and two days after, mice received 200ng pertussis toxin (List Biological Laboratories, via Quadragech) in 150µl PBS intravenously (i.v.).

- Prepare a solution of CFA by adding killed mycobacteria to a solution of IFA at a final concentration of 4mg/ml. Keep at 4°C.
- The MOG solution is prepared to be at 2mg/ml in PBS sterile, then aliquoted at 500ul or 1ml in eppendorf and kept frozen at -20°C.

**How to prepare CFA+MOG** (it is a 1 to 1 solution with CFA and MOG):

- Put the two solutions on ice.
- Usually I prepare 1.5ml CFA + 1.5ml MOG and preferentially use the small (3ml) glass syringes.  
Small syringes: by fixing a needle to one syringe, fill it up with the two solutions.  
Big syringes: by fixing a needle to one syringe fill it up with one of the two solutions, then fill up the other syringe with the other solution, by removing the plunger and blocking the end with one finger.
- Carefully connect the two syringes via the connector and make sure you do not introduce any air bubble!
- Mix a couple of times and put on ice. Repeat 5 to 6 times. If it too hot (summer) stay in the cold room.
- When there is no longer two phases it is ready. One of the tests is to put a drop of the CFA-MOG solution in water to see whether it disperses and dissolves (not ready), or stays clearly as one drop (ready!).
- Transfer the preparation in 1ml plastic syringe, put a blue needle (23G, 0.6x25mm) and keep in the fridge at 4°C (can stay for one or two months).

***Pertussis Toxin stock solution is at 1ug/10ul (=100ug/ml). > 2ul/mouse/150ul PBS  
4 mice = 10 ul Toxin + 750 PBS, 9 mice = 20 ul Toxin + 1500 PBS,***

**10 mice = 24ul Toxin +1800 PBS, 22 mice = 46ul Toxin +3600 PBS**

**Disease severity is scored daily on a 5 point scale:**

- 1- Tail atony; Loose end of tail tonus, but when hold by the tail mouse legs are opened (V) and mobile.
- 2- Hind limb weakness; Loose end of tail tonus, and when hold by the tail mouse legs are parallel (II) and mostly immobile.
- 3- Hind limb paralysis; Loose end of tail tonus, and when hold by the tail mouse legs are parallel (II) and mostly immobile, then when in the cage although keep 1 (or 2) paralyzed leg behind. Scrawl by pulling its body forward with front motility.
- 4- Quadriplegia; Loose end of tail tonus, and when hold by the tail mouse legs are parallel (II) and mostly immobile, then when in the cage although keep 2 paralyzed leg behind. Loose ability to pull its body forward with paralysis coming up to the front and creating weakness.
- 5- Moribund.