Induction and monitoring of chronic-relapsing experimental autoimmune encephalomyelitis in DA rats


Use autoclaved mortar, pestle, glass syringes, and bridges. All plastics should be sterile. The preparation can be done on a regular laboratory bench.

1. Preparation of the supplemented adjuvant
Weigh 30 mg of *Mycobacterium tuberculosis* (Difco catalog # 231141) and place into a mortar. Make into thin powder but without pressing too hard to avoid breaking the bacteria. Add 10 ml of complete Freund’s adjuvant H37Ra (Difco catalog # 231131) and mix. This supplemented adjuvant now contains 4 mg/ml *Mycobacterium tuberculosis*. Transfer to a tube.

2. Preparation of the spinal cord homogenate
Collect spinal cords from DA rats (age/gender indifferent) and store frozen at -80°C or use immediately. Weigh the frozen spinal cords to have enough to mix 1:1 (weight:volume) with the supplemented adjuvant. Mince the spinal cords as fine as possible with a razor blade, transfer to the mortar used in section 1 and make a paste with the pestle.

3. Preparation of the emulsion
The emulsion is a 1:1 mixture of antigen in aqueous solution added to supplemented complete Freund’s adjuvant. It is very important to add the antigen to the adjuvant and not the other way round!!! There is a lot of waste when making and injecting an emulsion, always prepare 1.5 or 2 times more than what you need.

Place supplemented complete Freund’s adjuvant in a tube and vortex at high speed. Add the spinal cord homogenate drop by drop while vortexing. When all of the spinal cord homogenate is added, vortex for 5 more minutes. The mixture should turn light pink.

Put the emulsion in a 5 ml glass syringe and link it to another 5 ml glass syringe using a 18G bridge (Fisher catalog # 14-825-17L). Send the emulsion from a syringe to the other until it becomes hard (5-10 min). If more than 5 ml of emulsion is prepared, use several 5 ml syringes, do NOT use larger syringes.

The emulsion can be stored in the syringes at 4°C for 3 weeks. I recommend preparing it at least 12 hours in advance to check that it does not break down, it should remain thick and not separate in 2 phases. The color will change overnight to a light yellow or beige.

4. Immunization of the rats
The recipients are 7-9 weeks old female DA rats. We get our rats from Harlan-Sprague Dawley.

Mix the emulsion in the syringes for a few minutes and transfer to the syringe used for the injection. Inject 200 μl subcutaneously at the very base of the tail using 23G needles and 3 ml Luer-Lock syringes under short-term anesthesia.

Rats should be observed twice daily and weighed daily (including weekends and holidays). Clinical signs are expected 7-15 days after injection of the emulsion.
5. **Clinical scoring**

0: no disease  
0.5: distal limp tail  
1: limp tail  
2: mild paraparesis, ataxia  
3: moderate paraparesis, the rats trips from time to time  
3.5: one hind limb is paralyzed, the other moves  
4: complete hind limb paralysis  
5: complete hind limb paralysis and incontinence  
6: moribund, difficulty breathing, does not eat or drink. **Euthanize immediately.**

Rats with a score of 3 or above need special care. We place food pellets and Gel packs (Napa Nectar) at the bottom of the cages. They must be monitored twice daily.

Rats with a score of 4 or above should be turned twice daily to avoid bed sores. They should be cleaned and treated with diaper rash ointment as needed to avoid urine scalds. It is also a good idea to offer water and wet food twice daily.

Rats with a score of 5 must be checked for dehydration and given sterile saline subcutaneously as needed.

Rats with EAE should not display pain. Pain (vocalizations, refusal to groom, eat or drink, automutilation, or any other sign of pain) is a criteria for immediate euthanasia.

6. **References**

