

Keywords: T cell, immunization, emulsion, Complete Freund's Adjuvant, EAE

1. Prepare immunization emulsions:
  - a. Prepare 200 ug of *M. tuberculosis* H37Ra (BD Difco Adjuvant, Fisher Scientific, Cat #DF0638-60-7; Waltham, MA) in 1x PBS or in 100 µg  $\alpha$ -syn<sub>32-46</sub> peptide (A&A peptides; San Diego, CA) dissolved in 1x PBS
2. Immunize 8- to 12-week-old male and female mice subcutaneously with 50 ul of emulsion into the chest area of the mouse, near the axillary lymph node at both front limbs for a totally of 100 ul of emulsion injected per mouse.
3. The day of immunization is designated as day 0.
4. At day 14, boost each mouse with the same immunization dose at the same site.
5. Administer intravenous injections of 400 ng of *B. pertussis* toxin (List Biological Laboratories, Cat #181; Campbell, CA) at 14- and 16-days post-initial immunization (day 0 and 2 post boost)
6. Monitor mice daily for their weight, food and fluid intake and neurological deficits and their weights were recorded daily. Mice are classified as sick if they displayed 12% reduction from 0 DPI weight and/or hunched posture with ruffled and ungroomed fur.