

## Preparation of Peritoneal Macrophages from Methylated BSA-Immunized Mice

(adapted from Cook *et al.* [2003] J. Immunol. 171:4816-4823)

### Methyl-BSA solutions:

- 1. Stock methyl-BSA solution:** Dissolve 20 mg methyl-BSA (Sigma A1009) in 10 ml sterile 0.9% NaCl (Sigma S8776).
- 2. Methyl-BSA in Complete Freund's adjuvant (CFA):** Place 2 ml of complete Freund's adjuvant (CFA; DIFCO 231131) that has been shaken well before use in a syringe. Place 2 ml of methyl-BSA solution in another syringe. Connect the 2 syringes to a double-hub micro-emulsifying needle (20 x 2-7/8 from Popper & Sons, Inc. [#7972]) and push back and forth ~ 30 times. The mixture becomes very viscous.
- 3. Methyl-BSA in Incomplete Freund's adjuvant (IFA):** same as above except use IFA (DIFCO 263910) instead of CFA.
- 4. Methyl-BSA in PBS:** mix 1 ml methyl-BSA with 9 ml sterile PBS.

### Immunization of mice:

- 1. Preparation of mice:** Anesthetize the mice with isoflurane using a volatile anaesthesia inhalation apparatus, with the oxygen flow level at 2 liters per minute and the isoflurane flow rate at 5%. Once the mice fall asleep, lower the isoflurane flow rate to 3%. Shave the area behind the tail using an Oster Finisher Trimmer (OS5910-01). Place the head of the mouse in a nose jar to keep anesthetized, making sure breathing is not compromised. Work with mice from only one cage at one time. In between the cages, clean the inhalation apparatus chamber with a wet towel.
- 2. Injections:**
  - a.** For i.d. injections, use a 0.5-cc "LO-Dose" V-100 insulin syringe with a 28½ G needle. Place mouse with the head facing toward you. Lift a small piece of skin with the injection needle. Have the bevel of the needle facing up. Keep the injection as shallow as possible.
  - b.** First injection: Inject two 50-microliter aliquots of methyl-BSA-CFA (100 µg), evenly spaced on each side of the tail. When correctly injected the vaccine will form a round, flat disc that is white in colour. If it appears pink or grey it has gone subcutaneous. To prevent scars or lesions at the site of injection, it may be necessary to treat the mice with iodine or another disinfectant on a daily basis.
  - c.** Second injection: as above at 14 days after the first immunization, but use methyl-BSA-IFA. For this injection, the fur will need to be shaved further away from the tail, so as not to inject into the same area.
  - d.** One week after the second i.d. injection, the mice are injected i.p. with 0.5 ml of methyl-BSA in PBS (100 µg). Four days after the i.p. injection, the mice are euthanized by CO<sub>2</sub> asphyxiation, and the peritoneal macrophages are harvested.