

**SOP No:** AHT 50

**SUBJECT:** MONOCLONAL (POLYCLONAL) ANTIBODY  
PRODUCTION (Ascites in Mice).

**POLICY** The *in vivo* production of antibodies by this method must be justified to the AEC. This technique is strongly discouraged by the NHMRC.  
The volume of pristane/Freunds complete should not exceed 0.2ml.  
A priming agent should not be used in individual mice more than once.  
No more than 2 survival taps should be attempted. A final tap can be performed at termination.  
Mice should be euthanased within 4 weeks of priming.

**PRECAUTIONS:** Gloves, eye protection, long-sleeve gown and closed in shoes.

**EQUIPMENT:** 25-27g needles  
70% Alcohol  
100µl and 5ml syringes  
collection tubes  
Pristane

**PROCEDURE:**

1. Prime adult female mice (> 6 weeks) by i/p injection of 0.2ml of pristane.
2. After 7 – 14 days, inject  $5 \times 10^5$  to  $5 \times 10^6$  hybridoma cells i/p in a volume of 0.1 – 0.5ml.
3. Tap the ascitic fluid when the abdomen becomes distended, using an 22g or smaller needle. (anaesthetising the mouse will improve the ease of collection and should be used for all recovery collections).
4. Replace lost fluid with 2 - 3ml of warm saline via S/C at multiple sites.

**RECOMMENDATIONS:**

Mice are monitored daily as per the score sheet.

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16.08.2012

  
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CHAIR OF AEC

**REFERENCES**

1. **Monoclonal Antibody Production (1999) Institute for Laboratory Animal Research.**
2. **NHMRC (2001). Guidelines on Monoclonal Antibody Production.**  
<http://.health.gov.au/nhmrc/research/awc/monosyn.htm>.
3. **Jennings VM (1995) Review of selected adjuvants used in antibody production. *ILAR Journal* 37(3): 119-125**