

# STANDARD OPERATING PROCEDURE #405

## MONOCLONAL ANTIBODY PRODUCTION - MICE

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### 1. PURPOSE

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This Standard Operating Procedure (SOP) describes the procedures for producing monoclonal antibodies in mice.

### 2. RESPONSIBILITY

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Principal Investigator (PI) and veterinary care staff.

### 3. MATERIALS

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- 3.1. Adjuvant
- 3.2. Antigen
- 3.3. Appropriate syringes and needles for injection
- 3.4. Blood collection materials
- 3.5. Scale
- 3.6. 70% ethanol
- 3.7. Sterile surgical instruments (forceps & scissors)
- 3.8. Container for ascites fluid collection (8 oz specimen container or 50 cc centrifuge tube)
- 3.9. Centrifuge tubes, 15 cc
- 3.10. Centrifuge
- 3.11. Wooden stir sticks
- 3.12. Transfer pipettes

### 4. CONSIDERATIONS

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- 4.1. The production of monoclonal in mice by the ascites method raises several issues of concern regarding the potential for severe and unnecessary pain and suffering for the animals. A number of *in vitro* replacements for the rodent ascites method of monoclonal antibody production have been developed. Every attempt should be made to obtain material already available or to use an *in vitro* method for production of monoclonal antibodies.
- 4.2. Before, producing monoclonal antibodies *in vivo*, the PI needs to justify to the Facility Animal Care Committee (FACC) in the Animal Use Protocol why *in vitro* production is not suitable. The following procedures can only be applied if the FACC accepts the justification and approves the *in vivo* production of monoclonal antibodies.

### 5. IMMUNIZATION PROTOCOL FOR PREPARATION OF HYBRIDOMA CELLS

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- 5.1. The antigen must be:
  - 5.1.1. Non-toxic
  - 5.1.2. Sterile
  - 5.1.3. Free of pyrogens
  - 5.1.4. pH within physiological limits
  - 5.1.5. Easily passed through a 25G needle

**NOTE:** Proteins in polyacrylamide gel may cause adverse reaction at the site of injection. Use another

- 5.2. For each scheduled immunization, prepare a sample consisting of a maximum of 50 micrograms of antigen in sterile PBS (or animal compatible buffer) in a volume of 50



