STANDARD OPERATING PROCEDURE #405

MONOCLONAL ANTIBODY PRODUCTION - MICE

1. PURPOSE

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

2. RESPONSIBILITY

Principal Investigator (PI) and veterinary care staff.

3. MATERIALS

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

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

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