The intent of this procedure is to describe monoclonal antibody production. This procedure is approved by the Cornell Institutional Animal Care and Use Committee (IACUC) and the Cornell Center for Animal Resources and Education (CARE). Any deviation from these recommendations should be scientifically justified and approved by the IACUC.

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1. Monoclonal Antibody Production

Background
In 1997 the National Institutes of Health issued, through their Office for Protection from Research Risks, some guidelines on monoclonal antibody production. This document states their expectation that \textit{in vitro} methods are to be used if at all possible and that a strong justification is provided should production occur \textit{in vivo} by the ascites method. Similarly, a panel put together by the National Research Council in 1999 concluded that \textit{in vitro} technology should be used whenever possible, although it admitted that under very specific circumstances \textit{in vivo} methods could still be used.

Monoclonal antibody production is a multi-step procedure:
1) Isolation of the cells that produce the antibody of interest. This is done by first immunizing a mouse or another animal with a specific antigen. Once antibodies are produced, B-cells, a type of white blood cells that is responsible for antibody production, are collected from the spleen. The specific cells that produce the antibody of interest are isolated \textit{in vitro}.
2) Fusion of antibody producing cells with cancerous cells. B-cells are then fused \textit{in vitro} with myeloma cells, a type of cancerous cells that are capable of dividing indefinitely, to form hybridoma cells. Hybridoma cells share properties of both types of cells and are therefore capable of repeated divisions and of secreting antibodies.
3) Growth of hybridoma cells. For the production of large amounts of monoclonal antibodies these hybridomas need to be grown and that can be accomplished by *in vitro* and *in vivo* methods:
   a) *in vitro* methods. Tissue culture technology is used to grow hybridoma cells that will secrete monoclonal antibodies in the culture media.
   b) *in vivo* methods. Hybridoma cells are injected into the peritoneal cavity of a mouse where they will multiply. As a result fluid is produced and collects inside the abdomen of the mouse. The more the hybridoma cells multiply the more fluid they produce. As the volume of fluid, known as ascites fluid, accumulates it distends the abdomen of the mice and causes discomfort and pain to the animal.

The use of the ascites method for the production of monoclonal antibodies requires that the IACUC conduct a thorough evaluation of the proposal to determine that there are no *in vitro* methods available for the production of the specific antibody. Once it is clear to the Committee that *in vivo* methods are the only ones available to the investigator, the main concern should be the minimization of pain and distress for the mice.

2. Review of Monoclonal Antibody Production Protocols by the IACUC

The IACUC recognizes that the use of the ascites method for the *in vivo* production of monoclonal antibodies might cause pain and distress to animals. The IACUC is aware of the existence of a variety of *in vitro* methods for the production of monoclonal antibodies. The IACUC encourages investigators to explore and use *in vitro* methods for the production of monoclonal antibodies whenever possible, the expectation being that *in vitro* methods will be tried by investigators before considering the submission of an application for the use of the ascites method in mice. The IACUC will not consider the use of *in vivo* methods unless scientifically justified.

When evaluating a submission from an investigator that requests the use of animals for the production of monoclonal antibodies, the IACUC will follow these procedures:
1) Assess if the production of monoclonal antibodies is scientifically justified based on the research objectives.
2) Evaluate the attempts made by the investigator at testing *in vitro* methods of monoclonal antibody production.
   a) Which *in vitro* systems have been used?
   b) Are *in vitro* systems appropriate for the amount of monoclonal antibody needed?
   c) Has use of a commercial source been considered?
   d) If cost is used as a determinant factor, what factors were included in cost estimates?
   e) Has *in vitro* production been tried without success?
3) If *in vivo* studies must be conducted, the IACUC should evaluate:
   a) Are *in vivo* pilot studies necessary?
   b) Are animal numbers properly justified?
   c) Are hybridomas tested for mouse antibody production?
d) Are procedures proposed for priming and tapping appropriate?
e) Are end points well defined and will animals be monitored adequately?


If no resources for in vitro monoclonal antibody production are internally available, several commercial companies offer their services for this purpose to investigators. Here is a brief listing of some of these companies (provided in alphabetical order). They offer both in vivo and in vitro services. The IACUC does not endorse any of these companies in any particular way, and it recognizes that other sources may be equally valid. Investigators are encouraged to consult these and other sources for their suitability for the specific research needs prior to the submission of animal use protocols for the use of the ascites method for the production of monoclonal antibodies.

**Cell Essentials Inc**
198 Tremont St./#181
Boston, MA 02116
ph: 617-636-2888, 888-929-6888
fax: 617-249-0888
website: [http://www.cell-essentials.com](http://www.cell-essentials.com)

**Covance Research Products**
Antisera Services
P.O. Box 7200
Denver, PA 17517
ph: 717-336-4921, 800-345-4114
fax: 717-336-3481
website: [http://www.covance.com](http://www.covance.com)

**Immuochemistry Technologies Llc**
9401 James Ave. South #155
Bloomington, MN 55431
ph: 612-888-8788, 800-829-3194
fax: 612-888-8988
website: [http://www.immunochemistry.com](http://www.immunochemistry.com)

**Primedica Inc**
2 Taft Court
Rockville, MD 20850
ph: 301-738-1112
fax: 301-738-1061
website: [http://www.primedica.com](http://www.primedica.com)

**QED Bioscience Inc**
11021 Via Frontera #203
San Diego, CA 92127
ph: 858-675-2405, 800-929-2114
fax: 858-592-1509
website: [http://www.qedbio.com](http://www.qedbio.com)

**Rockland Immunochemicals**
P.O. Box 316
Gilbertsville, PA 19525
ph: 610-369-1008, 800-656-7625
fax: 610-367-7825
website: [http://www.rockland-inc.com](http://www.rockland-inc.com)

**Taconic Biotechnology**
273 Hover Ave.
Germantown, NY 12526
ph: 518-537-6208, 888-822-6642
fax: 518-537-7287
website: [http://www.taconic.com](http://www.taconic.com)

**Protoprobe Inc**
10437 Innovation Dr. #328
Milwaukee, WI 53226
ph: 414-774-2670, 800-432-3711
fax: 414-774-0767
website: [http://www.protoprobe.com](http://www.protoprobe.com)
4. **Guidelines for the use of the ascites method**

**Priming agents.** Either one of these agents can be used:

1) Pristane, administered intraperitoneally 1 to 3 weeks before the implantation of the hybridoma tumor. A volume of 0.1–0.3 mL per mouse should be used, being recommended to start with a lower dose and never to exceed a volume of 0.3 mL.

2) Incomplete Freund’s Adjuvant, administered intraperitoneally, 24 hours before the implantation of the hybridoma tumor. Volume recommended of 0.25–0.5 mL.

Complete Freund’s Adjuvant must NOT be used since it causes a greater granulomatous reaction and its use is considered unnecessary and painful. The use of other conditions or other priming agents needs to be approved by the IACUC and should be scientifically justified in the proposal.

**Hybridoma testing.** Hybridoma cultures must be tested for mouse antibody production to detect the presence of viruses or mycoplasma before they are injected into animals. Contact CRAR for details.

**Hybridoma implantation.** Hybridoma cultures should be diluted to inject a total of $10^5$–$10^7$ cells in a volume that should not exceed 0.5 mL per mice.

**Animal Monitoring.** From the moment the animals receive the priming agents they should be monitored at least once a day for any sign of distress. Once the abdomen starts to distend the frequency should be increased to a minimum of two times each day.

Signs of distress in mice include any of the following: rapid breathing rate, slow, shallow or labored breathing, rapid weight loss, ruffled fur or rough hair coat, hunched posture, difficulty moving, hypothermia or hyperthermia, inappetence, diarrhea or constipation. If any of these signs is identified, veterinary staff needs to be informed for consultation and advice.

If these signs are ignored or if there is not appropriate and timely care the animal can become moribund and die. Moribund mice may show any of the signs of distress accompanied by any of the following: impaired ambulation (unable to reach food and water), evidence of muscle atrophy or signs of emaciation, lethargy (drowsiness, aversion to activity, lack of physical or mental alertness), prolonged inappetence, chronic diarrhea or constipation, bleeding, difficulty breathing, neurological disturbances, inability to remain upright. If any of these signs is identified veterinary staff should be notified immediately and the animal euthanized.

**Collection of ascites fluid.** Ascites fluid should be collected before abdominal distension causes distress to the mice, which in most cases occurs when the size of the abdomen reaches that of a late-pregnant mouse. The increase in body weight should not exceed 20% of the normal weight of age- and sex-matched animals of the same strain. Aseptic preparation is required to reduce the risk of contamination and peritonitis. Collection is accomplished by holding the animal in one hand and tapping the abdomen with a 20- to 22-gauge needle, allowing the fluid to flow freely into a collection tube. It is a good idea to anesthetize the
mice before tapping to reduce pain and distress associated with this procedure. Anesthesia and analgesia must be always used when training personnel on this procedure. The number of abdominal taps to a single mouse should be restricted to two, with a third one being possible only after euthanasia. Time between taps should be as short as possible and generally under 3 days. Mice should be kept under observation for one hour after each tap. If an animal shows signs of excessive distress or appears very debilitated after any of the taps it may be necessary to replace fluids or euthanize and veterinary staff should be contacted for immediate evaluation.

5. Relevant literature