



## CARE411.01 Monoclonal Antibody Production

The intent of this standard operating procedure (SOP) is to describe monoclonal antibody production and ascites fluid collection in mice. This SOP is intended for use by investigators and CARE staff involved in producing monoclonal antibodies. This SOP is approved by the Cornell Institutional Animal Care and Use Committee (IACUC) and the Cornell Center for Animal Resources and Education (CARE). Any exemption must be approved by the IACUC prior to its application.

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## 1. Introduction

Monoclonal antibodies (MAb) are antibodies which have a single, selected specificity and are usually produced continuously by 'immortalized' hybridoma cells. MAb are important reagents used in biomedical research, diagnostic methodologies (assays), and treatment of infectious and neoplastic diseases. Historically, there are two phases to MAb production in animals: the generation of B cells and mass production of MAb via the ascites induction method. The purpose of this document is to describe the methods by which ascites in mice is induced and the process by which ascites fluid is collected.

## 2. Materials

- Abdominal tap materials (e.g. 18-22 gauge needles, syringes, and antiseptic)
- Euthanasia materials (e.g. CO<sub>2</sub> chamber)
- Scale for weighing mice
- Fluids (normal saline or Lactated Ringer's Solution)
- A priming agent (i.e. Freund's Incomplete Adjuvant (FIA) or Pristane)
- Hybridoma cell suspension
- RADIL Submission Forms

### 3. Procedures

#### A. Animal Selection

Use mice of the same strain (syngenic) for immunization to produce the hybridoma clone and to subsequently produce a histocompatible monoclonal antibody (MAb).

**Note:** BALB/c mice are often the strain of choice, as many of the parental myeloma cells used in the fusion process are derived from BALB/c mice. The use of female retired breeders is advantageous due to previously stretched abdominal musculature.

#### B. Priming

- i) Prime adult female mice of at least 6 weeks of age or retired breeders.
- ii) Use a priming agent to prevent impairment of hybridoma cell growth and to cause serous fluid secretion in the peritoneal cavity.
- iii) Use Freund's Incomplete Adjuvant (FIA) or other priming agent such as Pristane.
- iv) Administer FIA into the peritoneal cavity (IP) once. Volume of IP injection is not to exceed 0.3 ml. If administering Pristane as the priming agent, do not exceed a volume 0.2 ml. IP (See [CARE SOP 401](#)-Recommended Volumes of Administered Substances).
- v) Wait 7-10 days before injecting the hybridoma cell suspension (obtained via in vitro cell culture propagation or in vivo)

#### C. Hybridoma Inoculation

- i) Test hybridoma cells for the presence of adventitious viral and mycoplasma agents prior to use of in vivo MAb propagation using the Mouse Antibody Production (MAP) test or PCR techniques. See [CARE SOP 619](#)-Tumor and Cell Line Testing.
- ii) Inject primed mice with up to  $3 \times 10^6$  hybridoma cells IP in a maximum volume of 1.0 ml. (See [CARE SOP 401](#)-Recommended Volumes of Administered Substances)
- iii) Monitor mice at least once daily for the first 7 days following hybridoma injection.
- iv) Once ascites is noted (abdominal swelling is typically apparent within 7-10 days post hybridoma injection), assess the mice at least once every 12 hours at ).

#### D. Ascites Tumor Growth

- i) Weigh mice daily beginning 4 days after inoculation to monitor progression of the ascites producing hybridoma. Document the weight of each mouse and retain for laboratory records.

**Note:** Do not allow mouse body weight gain to exceed 20% of the normal body weight of age- and sex-matched animals of the strain. Promptly address (e.g. call CARE veterinary staff) any other observations of unusual behavior or symptoms of pain or distress. See [CARE SOP 402](#)-Humane Intervention Points for further details.

#### E. Ascites Fluid Collection

- i) Collect ascites fluid for a maximum of two abdominal taps. Mice can be allowed to recover from the 1<sup>st</sup> tap; however, the second tap must occur subsequent to humane euthanasia.

**Note:** Fluid removal carries the risk of hypovolemic shock, hemorrhage, edema and death; thus, fluid replacement is necessary.

- ii) Administer 1-2 ml of replacement fluids (Normal saline or Lactated Ringer's Solution) subcutaneously prior to collection of large volumes of ascites fluid.
- iii) Perform the abdominal tap by inserting a hypodermic needle into the lower quadrant of the abdominal cavity with the hub of the needle at an approximate angle of 30 degrees.
- iv) Collect fluid that flows from the needle into a clean container via gravity. A maximum of 4-5 ml of ascitic fluid may be collected at the first (i.e. survival) tap.

**Note:** A large gauge needle permits rapid collection of the viscous ascitic fluid; however, needles larger than 20 gauge can cause tissue damage.

- v) Palpate the abdomen of the mouse to determine the presence of an intra-abdominal solid tumor.

**Note:** Humane euthanasia is indicated if a tumor is palpated; see CARE SOP 402, Humane Intervention Points..

- vi) For survival taps, monitor the mice closely for the first 60 minutes. Continue to perform ascites monitoring per sections 3,C,3 & 3,C,4 of this document.

**Note:** Treat signs of distress with humane euthanasia of the animal as per CARE SOP 301, Rodent Euthanasia.

## 4. Safety

- A. Follow CARE SOP 711 Sharps Precautions for handling and disposal of needles and syringes.

- B. Mice and their associated bodily fluids have the potential to incite allergic responses in humans; refer to the Cornell Occupational Health & Safety [Allergen Prevention document](#) for more information.
- C. If injured by an animal, see CARE 707, Animal Related Injury for appropriate actions.

## 5. Contingencies

Contact CARE veterinary staff for assistance with determination of humane endpoints.

## 6. References

- CARE SOP 401-Recommended Volumes of Administered Substances: <http://www.research.cornell.edu/care/documents/SOPs/CARE401.pdf>
- CARE SOP 619-Tumor and Cell Line Testing: <http://www.research.cornell.edu/care/documents/SOPs/CARE619.pdf>
- CARE SOP 402-Humane Intervention Points: <http://www.research.cornell.edu/care/documents/SOPs/CARE402.pdf>
- CARE 707-Animal Related Injury: <http://www.research.cornell.edu/care/documents/SOPs/CARE707.pdf>
- CARE SOP 301- Rodent Euthanasia: <http://www.research.cornell.edu/care/documents/SOPs/CARE301.pdf>
- Allergy Prevention: <http://www.research.cornell.edu/Care/documents/OHS/AllergyPreventionFactSheet.pdf>
- ILAR. "Immunization Procedures and Adjuvant Products. 46(3). 2005 pp.275-77
- Guidelines on Antibody Production; Canadian Council on Animal Care, Ottawa, Canada. 2002
- Guidelines-Monoclonal Antibody Production/Ascites Tumor Production, Office of Animal Care and Institutional Biosafety. Chicago, IL

## 7. Appendix

- A. Parameters affecting ascites production.
- B. Clinical, Pathophysiological, and Pathological effects of ascites production.

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## Appendix A

### Parameters affecting ascites production<sup>a</sup>

Parameter	Specification	Comment	Reference (see text)
Animal	Age	Highest Mab <sup>b</sup> concentrations in mice aged 43-75 days	Brodeur et al. 1984
	Sex	Males have a longer period of secretion, higher concentration/mL and greater volume of ascites, probably due to testosterone. Retired breeders.	Takakura et al. 1967
	Strain	Nude mice (irradiated mice) and SCID <sup>b</sup> mice can be used in case of nonsyngeneic hybridoma cells. Yields can be increased by use of BALB/c-derived cross-bred F1 hybrids.	<a href="http://iacuc.cwru.edu">http://iacuc.cwru.edu</a> Pistillo et al. 1992 Brodeur and Tsang 1986 Stewart et al. 1989
Hybridoma cells	Type of cell	Some cells show a large variety in growth pattern in animals and tend to grow poorly in some animals. Some cells have an aggressive growth pattern and are often poor MAb producers.	C.F.M.H., personal communication (see text) C.F.M.H., personal communication (see text); Brodeur et al. 1984
	Number of cells	Cell number affects the duration of secretion. Recommended number of cells is between $6 \times 10^5$ and $5 \times 10^6$ per mouse.	Johnson 1995 Brodeur et al. 1984
Primer	Product	By using FIA, <sup>b</sup> the interval between priming and hybridoma cell inoculation can be shortened and the animals survive more taps.	C.F.M.H., personal communication (see text)
	Volume	Optimum volume is 0.5 mL, but a lower volume (0.1-0.2 mL) has also been shown to be effective and to cause less distress.	C.F.M.H., personal communication (see text); Gillette 1987; FSU 1998

<sup>a</sup>Adapted from Hendriksen CFM, de Leeuw W. 1998. Production of monoclonal antibodies by the ascites method in laboratory animals. Res Immunol Forum 6 149:535-542.

<sup>b</sup>MAb, monoclonal antibody; SCID, severe combined immunodeficient; FIA, Freund's incomplete adjuvant.

## Appendix B

### Clinical, pathophysiological, and pathological effects of ascites production<sup>a</sup>

Clinical effects	Pathophysiological effects	Pathological effects
Abdominal distension	Anorexia	Peritonitis
Decreased activity and body mass	Anemia	Infiltrative tumor growth
Shrunken eyes	Dehydration	Adhesions in the abdomen
Difficulty with walking	Tachypnoe	Enlarged abdominal organs
Hunched posture	Circulatory shock	Blood in the abdominal cavity
Respiratory distress	Decreased venous, arterial, and renal blood flow	
Death	Ascites production	
	Immunosuppression	

<sup>a</sup>Adapted from Anon. 1989. Code of Practice for the Production of Monoclonal Antibodies. Rijswijk, The Netherlands: Veterinary Public Health Inspectorate.