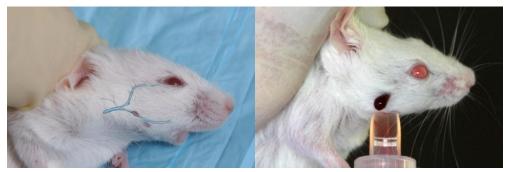
Animal use protocol sample: overview of the procedure for mouse hybridoma production

Hybridomas are permanent cell lines that secrete monoclonal antibodies. Animals, usually mice, are needed to produce these monoclonal antibody producing cells. Once these cells have been produced there are options for both in-vivo and in-vitro production of large volumes of the antibody.

Procedure. Mice are immunized with the antigen or agent by subcutaneous or intraperitoneal injection of up to 100 ul volume per site. In general, mice are immunized 2-3 times at 3 week intervals. A test blood sample is drawn after the 2nd or 3rd injection and tested by western blot or ELISA with a minimum dilution of 100-fold (investigators choice) considered sufficient. One (specify the number) additional immunization may be given if an antibody response is detected but the titer found to be below this limit. If the mouse does not respond to immunization after this time it is humanely euthanized.

Test blood samples are drawn from the large submandibular vein using the Goldenrod lancet (MEDIpoint, Inc. Mineola, N.Y.) modeled on the lancets used for humans. The lancet comes with specific point lengths for different size mice so the puncture is only as deep as the point of the lancet (Golde WT, Gollobin P, Rodriguez LL. A rapid, simple, and humane method for submandibular bleeding of mice using a lancet. Lab Anim (NY). 2005 Oct;34(9):39-43). This rapid method typically draws 4 to 10 drops of blood from the mouse, while causing minimal discomfort. All animals are in category I (category C for the UWYO form). To use the lancet, the mouse is restrained with one hand and the lancet used to make a quick puncture of the submandibular vein. Blood drops quickly form and are collected in a collection tube with up to 0.4 ml collected from a 25 gram mouse. If 100 ul or less of blood is collected, the blood draw can be repeated in 7 days, 200-300 ul requires a 2 week recovery period, and for 400 ul requires a 3 week recovery period before further sampling. A sterile gauze pad is applied to stop the bleeding if needed. After sampling the mouse is replaced in the cage and monitored for 15 minutes.

After the antibody response is found to be sufficient the mouse will receive a final immunization by intraperitoneal injection (or i.v. by tail vein, investigators choice) 3 days prior to euthanasia. The spleen is then removed and the splenocytes processed for in vitro hybridoma cell production.



Citations for Goldenrod lancet. Aging Cell. 2009 Jun;8(3):277-87. Epub 2009 Apr 9. Apoptosis. 2008 Aug;13(8):1005-12.

Standard Language approved by IACUC

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