

IRRADIATION PROTOCOL

The following is what we do using the Dartmouth Irradiation Facility for bone marrow chimerism using either pure C57Bl/6, C57Bl/6.SJL, or Rag^{-/-}γC^{-/-} animals (C57Bl/10XC57Bl/6).

Lethal irradiation of C57/B6 mice:

1050 Rads administered in a split dose (3 hrs. apart)

Mice loaded into pie as shown (on right) to immobilize them during irradiation (roughly 5 minutes each time)

*Minimum crude bone marrow for this protocol seems to be about 300,000 cells

Sublethal irradiation:

450 Rads single dose

Procedure:

At least 48 hours prior to desired irradiation time, contact Rendall Strawbridge to set up irradiation time. Specify mouse identity, number, dose and use of a Lucite pie.

Tag mice prior to irradiation (several days in advance is best).

After irradiation, cells can be injected immediately or up to 2-3 days later. Animals go into a barrier room.

For the first 2-4 weeks after the irradiation day, supplement the drinking water with Baytril: add 880 μL of stock solution as to 200 mL STERILE drinking water. Alternatively ARC can provide you with mouse chow with antibiotics in it. If your mice look anemic and cachexic, it will happen at day 13-19 and you can sometimes help them out giving IV saline or cut up apples in the cage.

Notes:

*Enrofloxacin (*Baytril*)

Fluoroquinolone antibiotic. Broad spectrum of activity. Recent publications have suggested that 5 mg/kg PO SID in dogs for sensitive *E. coli* infections, and 25 mg/kg PO SID in dogs and mice for sensitive *Staphylococcus aureus* infections are effective.

For sublethal OR lethal irradiation, we use 100mg/L or 20 mg per 200 mL sterile water; concentration of 0.001%.

Add 880 μL 2.27% Baytril to 200 mL sterile water (final concentration = 0.001%).
Replace water bottles with fresh Baytril water weekly

Lucite Pie from Braintree Scientific (www.braintreesci.com). Mouse pie cage (MPC) 21.5cm dia X 7.5 cm height, \$204. Needs to be thoroughly washed out afterward and sterilized (can autoclave in foil). Holds 11 mice.

