MAKING AVERTIN
WITH DAVID DANKORT

IMPORTANT NOTES

- Avertin is light sensitive – store in darkened bottle or wrapped in foil. If the stock solution yellows, replace it. A yellow solution is oxidized and can be quite toxic
- Avertin prepared as outlined is very stable. Stock solutions will remain good for many months at room temperatures.
- Storing the stock solution at 4° C. will cause the Avertin to precipitate out but will not harm the solution. You will have to redissolve the Avertin at room temperature before use, however.
- The working solution, if kept refrigerated and in a dark bottle, will be good for at least several months. We prepare 25-50 ml at a time and use it up before preparing new.

Materials Required:

10g 2,2,2,-tribromoethanol
Aldrich (**T48402**)
10ml Tertiary amyl alcohol (2-Methyl-2-butanol)
Sigma-Aldrich (Fluka 19954)

Sterilized dilutent:
(autoclave is best. alt: 0.22uM filtration)

50ul	1M	Tris (pH7.4)
25ul	0.5M	EDTA
1370 ul	5M	NaCl
48.75 ml		H_2O

---

1. Mix 10g 2,2,2,-tribromoethanol with 10ml tert-amyl alcohol. This can take a while – an end-over-end rotator works well. Some people will heat solution to speed the process – I do not recommend it as I am not sure if this hastens oxidation.

2. Mix 39.0 ml of diluent with 1.0 ml Avertin. Mix well overnight at 4°C works well.

3. Wrap in foil and store at 4°C.

**Avertin is lipid soluble so fat mice tend to take longer to be anesthetized. It is best to dose them first and wait to see when they become non-responsive.

4. Injection
0.016 X the body weight of the mouse (grams)**

weight (g)	volume (ml)
20	0.320
21	0.336
22	0.352
23	0.368
24	0.384
25	0.400
26	0.416
27	0.432
28	0.448
29	0.464
30	0.480
31	0.496
32	0.512
33	0.528
34	0.544

It will take the animal ~5 minutes to become fully anesthetized. An additional 0.1 to 0.2 ml of Avertin can be given as necessary. The animal will remain anesthetized for ~20 minutes.