



Title: *Buffers and Stock Solutions: Avertin Stock Solution*

No: RTLP-ALNY-8

Location:
Old CCRC Tripp Lab

Approval Date:
12 July 2005

Supersedes Date:

Materials:

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•Lab coat	•2% 2,2,2-tribromoethanol (Aldrich cat# T48402)	•Sterile Amber Serum vials 50 ml (Wheaton Science Products cat# 2223764)	•Sterile laminar flow hood
•Gloves	•2% tert-amyl alcohol (Aldrich cat# 152463)	•Sterile Bottle Stopper (Wheaton Science Products cat# 224100-202)	•Hot tap water
•Pipettes	•Warm plate with stirring capacity	Aluminum Seals (Wheaton Science Products cat# 224193-01)	Seal Crimper
•Pipetteman	•1L Amber Bottle		1.0 ml Syringes
•Pipetteman tips	4 Bottle top filer units .22um 500 ml (Nalgene # 450-0020)		15 Balb/c 3-5 wk old mice (Harlan)
•Pipette Aid			3 clean mice cages with bedding, food and water.
•Stir bar			

Procedure:

NOTE: Two liters of Avertin stock solution should be made each time- this will ensure that we have enough for a few months.

Solution preparation:

1. Prepare Avertin by adding 40mL of tert-amyl alcohol to a clean 1L Amber bottle with stir bar.
2. Add 20 g of 2,2,2-tribromoethanol to the alcohol.
3. Swirl until solid goes into solution at ambient temperature (this may take awhile).
4. Slowly add 500 ml warm ddH₂O to bottle on hot plate while stirring (hot plate on low).
5. Make sure salts stay in solution before you add more H₂O.
6. Bring this mixture slowly up to 1L.
7. Turn off hot plate when all of the solution has gone into solution..
8. Stir overnight at room temperature.
9. Check that 2,2,2-tribromoethanol has not precipitated out of solution. If necessary, turn hot plate back on until solid has gone back into solution.
10. Filter the Avertin solution with Bottle top filer units. Store avertin in cold room, 4 °C, in drawer out of light until batch is tested in mice.

Avertin Stock Solution Preparation

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In Vivo test of batch:

1. For each batch of Avertin made test 3-5 wk old 5 Balb/c mice with 0.200 cc . , 0.250 cc . or 0.300 Avertin. Use 5 mice per dose for a total of 15 mice.
2. Hold mouse in dominant hand by the scruff of neck and loop pinky-finger around base of the tail. Hold firmly and turn mouse so ventral side is facing upward.
3. Inject 0.200 cc . , or 0.250 cc . 0.300 Avertin solution into peritoneal cavity of mouse. Note how lightly mice are asleep by squeezing the hind feet.
4. Administer Avertin dose to all 15 mice and place into clean cages by dose group.
5. Check back the next day to see if any mice have not revived. Continue to check mice for at least 3 days to ensure survival.
6. Record % survivals by dose, and recommended dose for batch.

Aliquot Avertin:

1. Sterilely dispense 50 ml of avertin from 500 ml filter top bottle units into sterile Amber Serum vials in laminar flow hood.
2. Cap bottles with sterile Bottle Stopper, and Aluminum Seals
3. Crimp Aluminum Seals with crimping tool to seal bottle.
4. Label bottles as Avertin, 2,2,2-tribromoethanol with date of manufacture and initials.
5. Store at 4 °C until need.
6. Store with batch testing data - Record % survivals by dose, and recommended dose for batch, so that all users have access to info.

Author	Management Approval/Date	Effective Date