

Tel-Aviv University –Safety Unit

Standard Operating Procedure for 5-Bromo-2-Deoxyuridine (BrdU) in Animals

1. Health hazards	<p>5-Bromo-2'-deoxyuridine (5-BrdU) is a base analog of thymidine with the thymine substituted by bromouracil. This synthetic nucleoside incorporated into the newly synthesized DNA of replicating cells (during the S phase of the cell cycle), substituting for thymidine during DNA replication. 5-BrdU is routinely used to measure DNA synthesis and to label dividing cells.</p> <p>Because BrdU can replace thymidine, excessive exposure to the chemical can cause mutations. It is a halogenated compound considered to be a strong teratogen, a mutagen and anticipated carcinogen.</p> <p>BrdU has been demonstrated to be detectable over two years post-infusion.</p> <p>Toxicity: BrdU is cytotoxic and a potential mutagen and teratogen.</p> <p>Reproductive: BrdU can cause heritable genetic damage, harm to the fetus reproductive disorders and may alter genetic material.</p> <p>Primary routes of occupational exposure to BrdU include: inhalation (aerosol exposure), ingestion , dermal absorption, eye contact, and accidental injection.</p> <p>Target Organs: Immune system.</p> <p>* Pregnant and lactating women should avoid exposure to BrdU and animals that have been administered BrdU ,or use additional PPE (respirator).</p> <p>** As BrdU exposure can impair the immune system ;immunocompromised individuals should also use extreme caution when handling BrdU.</p>
2. Designated Area	ABSL-2 facility.
3. Training	Hazardous chemical training and training on this SOP is required before working with BrdU. This should include but is not limited to reviewing the MSDS, training on the physical hazards of the chemicals, symptoms of exposure, appropriate work practices, and proper use of PPE.
4. Personal Protective Equipment (PPE)	Double nitrile gloves, Chemical safety goggles, Lab coat and mask . Appropriate PPE should also be used for lower arms such as sleeve covers or securing gloves over the sleeves of laboratory coat. Personnel should not work with BrdU if skin is cut or scratched.

5.General . Precautions for Animal Use	Tools (as, syringe, blades and safety needles where possible) should be adapted for BSL-2. Have a sharps container in close vicinity. Animals should be restrained or anesthetized during injection.
6. Environmental / Ventilation Controls	The preparation of BrdU including reconstitution, weighing, and diluting should be performed in a fume hood or biological safety cabinet (class II Type B). Work should be done over absorbent pads. Work should be conducted in ABSL-2 facility, over absorbent pads in a class II type A1 or A2 biological cabinet.
5. Special Handling Procedures & Storage Requirements	Handling: BrdU should be handled in containment and done over absorbent pads. Utilize safe sharps procedures (i.e. sharps container in the immediate vicinity, Leurlock syringes are recommended). The fume hood or other approved containment must be cleaned upon completion of tasks. When transporting BrdU, the vials should be placed in secondary, sealed, plastic, labeled, non-breakable containers. Storage: Keep container tightly closed. Store in -20°C.
8. Precautions for Animal Use	No recapping needles. Have a sharps container in close vicinity. Animals should be restrained or anesthetized during injection. Once BrdU is injected, animals , animal waste and cages are considered hazardous for a minimum of three days.
7. Animal handling practices	1. Animals must be housed in filter top cages marked as biohazards (including the name of the pathogen/biohazard). Handling the cages (including bedding) will be done only by the researchers. 2. Use a class II Biological Safety Cabinet at all times (especially during injection or any surgical procedure), when performing work on these animals and/or when moving animals from dirty to clean cages. 3. Injecting animals with BrdU: Animals will be injected IP with BrdU within Class II Biosafety cabinet or designated chemical fume hood. All needles will be disposed of in sharps container – do not recap or bend needles. 4. Infected animals considered hazardous for a minimum of three days after each administration of BrdU; take precautions to avoid the creation of aerosols when changing or washing cages, or cleaning the room. A respirator is recommended for personnel that are immunocompromised or pregnant and for healthy personnel if work is done outside the ventilated cabinet .

5. Care should be taken to avoid exposure to bedding dust when handling exposed animals and their waste materials during this time.
6. Dead animals must be placed in primary plastic bags, which are then placed in biosafety bags for infectious waste incineration.
7. All surfaces and racks that may be contaminated will be decontaminated with detergent solution followed by water ASAP.
8. The first cage change after each drug administration is to be done no sooner than 3 days after the administration. The bedding is considered contaminated and requires special handling.

When changing cages, use the following technique:

- Transfer the animals to clean cages .
- Insert the used cages in a plastic bag .
- Twist the ends of full bags, and seal with tape. Label with wide tape or other type of label marked “toxin-BrdU ”.
- Transport the bags of cages to a HEPA filtered dumping station that draws air away from the use. .(it is recommended to use a mask) or fume hood.
- All contaminated bedding will be labeled as hazardous materials and handled accordingly :
incinerated or placed in chemical waste bags for disposal.
- Animal carcasses will be incinerated or handled as Chemical waste.
- After this first cage change there is no need for further special precautions to be taken regarding
the animals or the cages as long as the animals have not received any more BrdU.
- The cages should then be put in plastic bags (marked “toxin-BrdU) and sealed for transport to the washroom.
- In the washroom ,cages should be unloaded from the bags with the appropriate PPE as mentioned above and run through the cage wash in the conventional manner. Note- cage wash personnel that meet the criteria for extra precautions above (pregnant exc.) should take extra precautions (additional PPE) when handling cages that may have BrdU contamination.

<p>9. Spill and Accident Procedures</p>	<ol style="list-style-type: none"> 1. Spills must be cleaned immediately by properly protected trained personnel. 2. Liquid Spills: should be cleaned immediately by personnel wearing a gown, goggles, two pairs of gloves (nitrile). Use absorbent pads to wipe liquid. The spill area should then be cleaned thoroughly with a detergent solution followed by clean water. Place waste in plastic bag and then in the chemical waste container. 3. Powder Spills: should be cleaned immediately by personnel wearing a gown, goggles, two pairs of gloves (nitrile). For powder spills outside of a fume hood or approved containment, non-essential personnel should be instructed to leave the laboratory and entrance should be restricted. In addition to the above specified PPE, A respirator should also be worn. The spill area should then be cleaned thoroughly with a detergent solution followed by clean water. Place waste in a plastic bag and then in the chemical waste container. <p><u>Exposure:</u></p> <ol style="list-style-type: none"> 4. In case of skin contact or injection with BrdU, wash the affected area with soap and water for at least 15 minutes. Consult with Employee Health Center. 5. For eye exposure, flush with water for at least 15 minutes. Consult with Employee Health Center,. Report incident to supervisor. Supervisor reports the accident/injury to the Biosafety Unit.
<p>10. Waste Disposal</p>	<p>Dispose all waste material in the appropriate chemical waste container.</p> <p>BrdU has a chemical waste characteristic of persistence. Any full strength unused reagent , any mixtures containing 0.01% or more of BrdU , and any spill clean-up debris should be managed as chemical waste.</p>
<p>I hereby confirm that I have read the SOP (Standard Operating Procedure) for Working with 5-Bromo-2-Deoxyuridine (BrdU) in Animals, and agree to follow these procedures.</p>	
<p>Name:</p>	<p>Title:</p>
<p>Signature:</p>	<p>Date:</p>

Dr. Esther Michael - Biological Safety Office, : 640-9966

