



## **Protocol for drug testing in *T. brucei* acute phase in infected mice**

### **Experimental animals**

Female Balb/c mice, 5 weeks old (20 - 25g). Groups of five mice are divided in control (vehicle treated) and the different groups of drug treatments.

### **Transgenic parasite line**

The transgenic *T. brucei brucei* ANTAT1.1 expressing *Renilla* luciferase is used. The parasite was transfected with RLuc-pHD309 plasmid and gently given by Dr. Nick Van Reet. (Claes, F. et al, 2009. *PLoS Negl Trop Dis.* 2009 Jul 21;3(7):e486. PMID: [19621071](#))

### **In vivo development of acute phase + Drug treatments**

Groups of 5 mice are infected via i.p. injection with  $5 \times 10^4$  *T. brucei brucei* ANTAT1.1- Luc bloodstream forms from culture in HMI-9 medium. Three days after infection the mice were anesthetized by inhalation of isoflurane (controlled flow of 1.5% isoflurane in air was administered through a nose cone via a gas anesthesia system). Mice were injected intraperitoneally with 100 uL of a coelenterazine native solution (5uL from 2 mg/ml stock [nanolight] dissolved in methanol + 95 uL of sterile PBS). Mice were imaged for 45 seconds, 5 to 10 min after injection for of coelenterazine with an IVIS 100 (Xenogen, Alameda, CA) and the data acquisition and analysis were performed with the software LivingImage (Xenogen). One day later (4 days after infection) treatment with compounds at the desired dose or vehicle control is started by i.p. injection or oral gavage. After treatment, mice were imaged again after anesthesia and injection of coelenterazine as described above. The ratio of parasite levels is calculated for each animal dividing the luciferase signal on day 9 (after 5 days of treatment) by the luciferase signal on day 3 (before treatment starts on day 4). Pentamidine (5mg/kg/day) or Suramin (40mg/kg/day) can be used as positive controls diluted in sterile PBS and administered by i.p. injection for 5 days.