Recommended Standard Methods of Blood Collection: *Rats*

Method	Maximum volume *(per sample)*	Anesthesia Required	Description	Special Considerations
Tail Vein	up to 2 mL		Following collection site cleaning with 70% alcohol, the rat is restrained *(state method of restraint)* and blood is collected using a 21-23 gauge needle from the lateral tail vein. Blood flow is stopped by applying pressure with sterile gauze to achieve hemostasis.	• No more than eight blood samples should be taken per session and in any 24-hour period. • Rats may be warmed to promote blood vessel dilation, however, animals must be closely monitored during the period of heat exposure.
Saphenous Vein	Up to 0.2 mL		The rat is restrained *(state method of restraint)* and the hind leg is immobilized in the extended position by applying gentle downward pressure immediately above the knee joint. The collection site is shaved with an electric clipper and cleaned with three alternating scrubs of 70% alcohol and betadine. Blood is collected using a 23 gauge needle from the lateral saphenous vein. Blood flow is stopped by applying pressure with sterile gauze to achieve hemostasis.	• No more than four blood samples should be taken within any 24-hour period. For multiple samples the scab or the blood clot should be removed.
Retro-Orbital Plexus	Up to 4 mL with recovery; 4-10 mL non-survival	YES	Following anesthesia and restraint, the neck is gently scruffed and the eye made to bulge. A sterile capillary tube/pipette is inserted medially, laterally or dorsally. Blood is allowed to flow by capillary action into the capillary tube/pipette. Blood flow is stopped by applying pressure with sterile gauze over the closed eyelid for approximately 30 seconds to achieve hemostasis.	• Individuals performing retro-orbital blood collection must be adequately trained due to the potential for significant complications. • It is recommended that only one sample be taken. No more than one bleed per eye should be taken - where a second retro-orbital bleed is required this should be performed as a terminal procedure. • Adequate time should elapse between sampling to allow peri-orbital tissue repair to take place. An interval of two weeks between bleeds should allow damaged tissue to repair in most cases.
Cardiac Puncture *(Non-survival)*	Up to 15 mL	YES	The rat is anesthetized and blood is collected via the left ventricle using a 19-21 gauge needle. Blood will be withdrawn slowly to prevent the heart from collapsing.	• Ensure euthanasia of the animal post-procedure.

NOTES:

Total blood volume of a rat is 64 ml/kg or 6.4 % of total body weight (BW).

As a general rule, 20 drops = 1 mL (i.e. 5 drops = 250 uL)

Recommended Standard Methods of Blood Collection: *Rats*

NOTES: *(cont'd)*

No more than 1% of the animal's blood volume in one collection or over a 24 hour period.

No more than 7.5% (0.48 ml/ 100 g BW) of total blood volume can be collected in a single or multiple draws over a week period.

If 10 % (0.64 ml/ 100 gm BW) of total blood vol, must allow 10 days recovery before next draw.

If 15 % (0.96 ml/ 100 g BW) of total blood volume is collected, must allow 15 days recovery before next draw.

If multiple samples are needed in a 24 h period, alternate collection Alternate legs for saphenous vein, R and L dorsal tail veins.