

Recommended Standard Methods of Blood Collection: *Mice*

Method	Maximum volume (per sample)	Anesthesia Required	Description	Special Considerations
Tail Vein	up to 0.2 mL		Following collection site cleaning with 70% alcohol, the mouse is restrained (<i>state method of restraint</i>) and blood is collected using a 25-27 gauge needle from the lateral tail vein. Blood flow is stopped by applying pressure with sterile gauze to achieve hemostasis.	<ul style="list-style-type: none"> • One or two blood samples can be taken per session and in a 24-hour period, depending on sample volume. • Mice 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 mouse is restrained (<i>state method of restraint</i>) 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 25-27 gauge needle from the lateral saphenous vein. Blood flow is stopped by applying pressure with sterile gauze to achieve hemostasis.	<ul style="list-style-type: none"> • No more than four blood samples should be taken within a 24-hour period. For multiple samples the scab or the blood clot should be removed.
Submandibular Vein	up to 0.2 mL		The mouse is restrained (<i>state method of restraint</i>) and the hairless freckle on the side of the jaw is located. An 18 gauge needle is aligned so that it is pointing at the far side of mouse's face, at the base of the far ear pra the base of the far side of the mouth. The freckle is pricked with the needle, only up to the depth of the bevel. Blood is collected with a collection tube, and blood flow is stopped by applying pressure with sterile gauze to achieve hemostasis.	
Tail Snip (<i>conditionally acceptable with justification</i>)	10 uL		The mouse is restrained (<i>state method of restraint</i>) and the tail is cleaned with three alternating scrubs of 70% alcohol and betadine. Using a sterile scalpel blade, no more than 1 mm of tail tissue is clipped. Blood is collected by gently milking the tail. Blood flow is stopped by applying pressure with sterile gauze to achieve hemostasis.	<ul style="list-style-type: none"> • A local anesthetic (e.g. EMLA cream) can be applied to the tail tip 30 minutes prior to blood sampling. • Normally no more than a maximum of four samples should be taken in any 24 hour period. • Where multiple samples are collected this should be done by removing the scab or blood clot from the tail tip.

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Retro-Orbital Sinus	up to 0.2 mL	YES	Following anesthesia and restraint, the neck 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.	<ul style="list-style-type: none"> • Individuals performing retro-orbital blood collection must be adequately trained due to the potential for significant complications. • Retro-orbital blood collection must include the administration of an antibiotic ophthalmic analgesic ointment to both eyes to minimize pain and distress and prevent desiccation and infection. • Sufficient time must be provided to allow the eye to heal before it is re-used for blood collection. This generally requires 4 weeks for recovery to minimize pathology in orbital tissues, including hemorrhage, inflammation, and infection.
Cardiac Puncture (<i>Non-survival</i>)	up to 1 mL	YES	The mouse is anesthetized and blood is collected via the left ventricle using a 23-25 gauge needle. Blood will be withdrawn slowly to prevent the heart from collapsing.	<ul style="list-style-type: none"> • Ensure euthanasia of the animal post-procedure.

NOTES:

Total blood volume of a mouse is 75 ml/kg or 7.5% of total body weight or 0.75 ml/10 g body weight (BW).

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

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

No more than 7.5% (0.056 ml/ 10 g BW) of total blood volume can be collected in a single or multiple draws over a week period.

If 10 % (0.075 ml/ 100 gm BW) of total blood vol, must allow 10 days recovery before next draw.

If 15 % (0.11 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.