



## **USDA ARS National Animal Germplasm Program**

### **Whole Blood Cryopreservation**

#### **Required supplies:**

- Purple top blood tubes containing EDTA
- Dimethylsulfoxide (DMSO)
- Serological pipettes
- Cryovials (1.5 or 2.0 mL with gasket lids)
- Labels for cryovials with National Animal Germplasm Program (NAGP) identification numbers
- Cardboard cryo-box
- Inventory sheets from NAGP
- Shipping containers with UPS/FEDEX labels approved by NAGP.

#### **Instructions for whole blood cryopreservation:**

Label cryovials (4 per animal) with the NAGP identification number.

Collect blood into purple top blood collection tubes containing EDTA. NOTE: Blood can be stored at 5 °C for up to 7 days prior to freezing but minimal holding time results in optimal post-thaw sample quality.

Measure the sample volume and add DMSO to a final volume of 10% in the purple top tube. Separate pipette tips must be used for each sample.

Close the sample tube, gently mixed, and transfer aliquots (1.0 mL) into 4 cryovials per animal.

Loosen the cap of the cryovials and place them into the cardboard cryo-box supplied by NAGP.

Record the box identification number and location of the sample within the cryo-box on the inventory sheet provided by NAGP.

Place the cryo-box, without the lid attached, in a -20 °C freezer for approximately 2hours.

Once frozen, secure the caps and the lid of the cryo-box and maintain the samples in the freezer until shipping.

#### **Overnight shipping:**

The samples are shipped overnight to NAGP using one of the following approved shipping containers:

Dry shipper: place the cryo-box directly into the shipper, include crumpled newspaper or some other type of padding, if necessary, and secure the plug and lid of the shipper.

Impact Shipper: the samples in cardboard cryo-boxes are placed into the shipper and should be surrounded by reusable ice blocks and sealed according to the Impact Shipper Protocol available on the Animal GRIN webpage noting the specific temperature for each species and type of tissue:

<https://www.ars.usda.gov/plains-area/fort-collins-co/center-for-agricultural-resources-research/paagrpru/docs/animal/animal-protocols/>

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