

Information on Sampling and Preservation of Sample Material for **Genetic Analysis**

1 General Information on Sampling

A professional collection and preservation of sample material is of decisive importance for successful genetic analysis. To avoid contamination with external DNA, sample collection should always be done with the highest care. Therefore, the following basic rules should be observed:

- A. Use disposable gloves during all steps of sample collection to avoid contamination and to protect you from zoonotic diseases transmitted, for example, through scat samples from wild animals. Change gloves between each sample collection event.
- B. Replace or thoroughly clean all sampling materials after each sampling. Disposable forceps, scalpels, etc. is preferably used. If disposables are not available, any sampling material must be cleaned with water and ethanol and subsequently flamed (e.g., with a gas burner) before reuse.
- C. Preserve collected materials as soon as possible, e.g., through drying, freezing, or 96% ethanol. Do not send any samples in an unpreserved state. The optimal preservation strategy depends on the type of sample collected. See next page for specific information various on sample types. Alternative preservation methods should be clarified with us before use.

From our experience, conscientious observance of the instructions for the collection and preservation of samples will increase analysis success. After consultation, we can send suitable collection materials. Please do not hesitate to contact us if you have any questions regarding the collection and treatment of sample material.

Contact - Centre for Wildlife Genetics

Phone: +49 6051-61954-3138 wildtiergenetik@senckenberg.de Email:

2 Ordering and Shipping Information

Please visit our order management system www.wildtiergenetik.de to create a user account and place orders for DNA analyses. Samples sent to us will only be processed if a correct order has been placed! Please make sure that the number of samples and the information on the samples exactly match the information provided in the online order, otherwise delays in the process may occur. After sending the online order, the samples can be sent to the following address:

Senckenberg Centre for Wildlife Genetics Probenannahme Clamecystraße 12 63571 Gelnhausen GERMANY



Ordering information for supplies in Germany

- ¹ Medishop, plastic container 70 ml yellow, SAR759922721
- ² Carl Roth, FTA®-Cards, CL93.1
- ³ Carl Roth, Rotilabo[®]-sample bags, P280.2 ⁴ Carl Roth, Silica gel orange - Desiccant Sachets, N077.2
- ⁵ Carl Roth, FTA[®]- Cards with colour indicator, CL94.1
- ⁶ Carl Roth, Rotiprotect-Disposable gloves, L950.1
- ⁷ Carl Roth, Rotilabo[®]-Circular filters AP80.1
- ⁸ Carl Roth, Cutfix[®] Scalpel sterile, X006.1
 ⁹ Carl Roth, Rotilabo[®]-Tweezers disposable, sterile, KL05.1
- ¹⁰Carl Roth, Ethanol 96 % (extra pure), P075.3
- ¹¹ Sarstedt, Forensic swab with ventilation membrane, ISO
- 18385, 95, EH12.1, Order number: 80.629.001
- www.medishop.de, www.carl-roth.com

Left: Sealed plastic cup with 33 ml undenatured ethanol (96%) for the preservation of scat material or muscle tissue. Middle: Ziplock bag for storing hair samples with filter paper and desiccant. Right: Sarsted Forensic Swab for saliva swabs of livestock predators, also suitable for saliva or blood samples.

3 Specifications for sampling and preservation of different sample types

NONINVASIVELY COLLECTED SAMPLES often show low DNA quality and quantity and need special care during sampling and lab analysis. Special DNA extraction protocols and performance of multiple analysis replicates cause increased analysis costs.

Hairs should be placed in a folded filter paper⁷ and then stored in a ziplock bag³ with desiccant⁴ at room temperature (see illustration overleaf). If needed, dry hair material may also be shipped without desiccant. The chance for a successful genetic analysis increases with the number of (dry) hairs with roots. Please avoid to collect hairs on adhesive surfaces, as this can have negative effects on the analyses. Collecting hairs on adhesive strips etc. is only recommended in exceptional cases (e.g., when searching areas for hair and tissue remnants, e.g. in forensic examinations).

Scats (Feces) are placed in plastic containers¹ filled with approx. 33 ml undenatured ethanol¹⁰ (96%; see figure). The ethanol should completely cover the sample. Store at room temperature or in the refrigerator. For longer storage times (several months to years), storage at -20°C is recommended. <u>Caution</u>: larger quantities of sample containers must only be stored in explosion-proof refrigerators/freezers. Ideally, a sterile object should be used to collect scats (scalpel, tweezers, cleaned and flamed blade of a pocket knife; a wooden stick is also suitable; this prevents cross-contamination via an unclean blade if no suitable cleaning facilities are available). In the case of larger scats, a piece of 3-5 cm should be cut off one of the ends and placed in the ethanol-filled sample container. A shiny mucus layer of the intestinal mucosa is present on the surface of the exudate. The mucus layer is particularly suitable for DNA analysis and should be transferred to the sample container. The container should be labelled with a pencil (avoids smudging).

Urine is ideally collected in winter in the snow. Urine samples are collected in plastic containers¹ filled with 96% ethanol (no freezer bags or similar) and stored at room temperature. If no ethanol container is available, the urine should be frozen in the snow and sent to us cooled. **Small traces of body liquids**, such as urine or blood, can be collected with one or two swabs¹¹. Let the frozen liquids melt on your hand covered with a disposable glove and collect with the swab (see also section "Blood" below).

Predator identification from kills is done by collecting saliva traces from the predator from throat bites and surrounding areas, or wound edges with Sarstedt Forensic swabs with ventilation membrane¹¹. The swab is rubbed back and forth several times at the appropriate area and also rotated around its own axis to collect as many DNA traces as possible. Swabs may be moistened with 1x TE buffer (alternatively use sterile water) before sampling. Due to the high risk of contamination we recommend to order buffer produced in our laboratory or equivalent products from specialist suppliers. After sampling, the swab is stored in the tube. If no forensic swab is available, sterile cotton swabs can be used. After sampling, the swab is dried with the ziplock bag open or outside the bag (especially if the moisture content is high). Dried swabs are placed in a folded filter paper⁷ and stored in a ziplock bag³ with desiccant⁴ at room temperature. Caution: Successful predator identification may require taking several swab samples in addition to the primary sample from the throat bite. All collected samples from a kill should be stored separately to avoid cross-contamination. Apart from our recommended items sterile swab tubes offered by other manufacturers for forensic analyses are equally suitable for sampling, if desiccant or or an alternative drying mechanism. Samples are ideally taken within 24 h after the kill incident, as the probability of a successful predator identification decreases over time. Dry and cold weather has a favorable effect and can cause a longer preservation of DNA traces on the carcass. Please send in samples as soon as possible.

Bones and **teeth** of carcasses are placed in a ziplock bag³ with desiccant⁴ and stored at room temperature.

INVASIVE SAMPLES normally contain large amounts of DNA but may require direct handling of the animal

Tissue (ideally muscle tissue the approx. size of an olive) is placed in a plastic container¹ filled with 33 ml undenatured ethanol¹⁰ (96%; see illustration overleaf) and stored at room temperature. For longer storage times (months to years), storage in the freezer is recommended. Caution: larger quantities of sample containers may only be stored in explosion-proof freezers. If no suitable sample containers or 96% ethanol are available, tissue samples may also be stored in a container with other kinds of high-percentage alcohol. The containers should be checked for leaks before shipment. In exceptional cases, tissue samples can be frozen directly and submitted for genetic analysis while maintaining the cold chain. Containers are ideally labelled with a pencil (avoids smudging).

Blood should be collected with Sarstedt Forensic swabs with ventilation membrane¹¹ and can be stored at room temperature (see figure overleaf). If no forensic swab is available, sterile cotton swabs can be used. Dried swabs are placed in a folded filter paper⁷ and stored in a ziplock bag³ with desiccant⁴. For sampling dried blood traces, it is recommended to moisten the swabs with 1x TE buffer beforehand. Alternatively, a few drops can be dripped onto the field of an FTA card² and then dried. The card is then placed in a ziplock bag³ with desiccant⁴ and stored at room temperature. If no cotton swabs or FTA cards² are at hand, blood samples can also be collected in the field in commercially available EDTA (anticoagulant) tubes, as is common practice among veterinarians. The samples must then be sent <u>immediately with cold storage</u>. Alternatively, blood samples (up to a maximum of 3 ml) can be placed in containers with 33 ml of min. 96% ethanol¹⁰, stored at room temperature and shipped. If no collection materials are available, blood may be collected with a tissue or piece of paper and dried subsequently before sending within a ziplock³ bag.

Saliva/ Buccal swabs are preferably collected using Sarstedt Forensic swabs¹¹ by rubbing them for several seconds against the inner mouth parts. Alternatively, sterile cotton swabs are stored in ziplock bags³ with filter paper and desic-cant (see above).

In special cases, e.g. an animal attack on humans, please contact us prior to taking samples!