

Information on sampling and preservation of sample material for genetic analyses

1 General information on sampling

The collection of samples as well as the professional and timely preservation of the collected samples are of decisive importance for successful genetic analysis. To avoid contamination with foreign DNA, sample collection should always be carried out with the highest care. Therefore, the following basic rules should be observed:

- A. Disposable gloves should be worn during all steps of sample collection. These serve not only to avoid contamination but to protect against pathogens that can be transmitted, for example, by scat samples from wild animals. Gloves should also be changed during sampling if contaminated packaging, pens or other objects have been touched. After sampling is completed, the gloves should be disposed of. Changing gloves between each sampling, e.g. also when sampling a clawed prey animal several times, is strongly advised.
- B. After each sampling, all materials used that have come into contact with the sample material must be replaced or thoroughly cleaned. The use of disposable forceps, scalpels, etc. is always preferable. If disposables are not available, any sampling material must be cleaned with water AND ethanol then additionally flamed (e.g. with a gas burner) before reuse.
- C. After sampling, the samples should be preserved as soon as possible, e.g. by drying, cooling or placing in 96% ethanol. Sample material should never be sent in an untreated state. Depending on the type of sample, certain preservation methods are more suitable (cf. par. 3). Alternative preservation methods should be clarified with us before use.

Conscientious observance of the instructions for the treatment of samples leads, according to our many years of experience, to an increase in success rates and better results. After consultation, we can compile and send you suitable collection material. Please do not hesitate to contact us if you have any questions or uncertainties regarding the sampling and treatment of sample material.

Contact Center for Wildlife Genetics

Phone: **+49 6051-61954-3138**

Email: **wildtiergenetik@senckenberg.de**

2 Ordering and shipping information

The ordering of genetic analyses is done via an online order management system (www.wildtiergenetik.de) and requires the creation of a user account. Without a created order, samples sent in cannot be processed. Please make sure that the number of samples and the information on the samples exactly match the information in the online order, otherwise delays in the process may occur. After creating the online order, the samples can be sent to the following address:

**Senckenberg Gesellschaft
Zentrum für Wildtiergenetik
Probenannahme Wildtiergenetik
Clamecystraße 12
D-63571 Gelnhausen**



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
- ¹⁰ Carl Roth, Ethanol 96 % (extra pure), P075.3
- ¹¹ Carl Roth, Cotton buds, wood, sterile, EH12.1

www.medishop.de, www.carl-roth.com

Left: Sealed plastic cup with 33 ml undenatured ethanol (96%) for preservation of excrement or muscle tissue.

Right: Ziplock bag for storing hair samples with filter paper and desiccant and sample labelling. Also suitable for saliva or blood samples and saliva swabs of livestock predators.

3 Specifications for sampling and preservation of different sample types

Kill swabs are taken at suitable locations (throat bite, area surrounding the throat bite, wound edges) with sterile cotton swabs¹¹ in order to obtain saliva samples of the causative agent from presumably killed animals. The swab is rubbed back and forth several times at the appropriate area and also rotated around its own axis to collect as much DNA/saliva as possible. The swabs should be moistened with sterile water or, better, 1x TE buffer immediately before sampling (due to the high risk of contamination, we recommend purchasing the buffer produced in our laboratory or equivalent products from specialist suppliers). After sampling, the swab is dried with the ziplock bag open or outside the bag (especially if the moisture content is high). It is then placed in a folded filter paper⁷ and stored in a ziplock bag³ with desiccant⁴ at room temperature. Caution: There is an increased risk of contamination for kill swabs. It is recommended to take samples from other sites (see above) in addition to the throat bite to increase the chance of a successful analysis (please use new swabs and store them separately). The sterile swab tubes offered by various manufacturers for forensic analyses are also suitable for sampling (before purchasing, make sure that a drying agent or mechanism is available). It is important that sampling is done within 24 h after the kill incident, if possible, as the probability of a successful determination of species and individual steadily decreases over time. Dry and cold weather has a favourable effect and can cause a longer preservation of DNA traces on the carcass. Please send the sample to our lab as soon as possible.

Hair should be placed in a folded filter paper⁷ and then stored in a ziplock bag³ with desiccant⁴ at room temperature (see illustration overleaf). If necessary, hair can also be shipped in a dry state without desiccant. It should be noted that the chance for a successful genetic analysis increases when a larger number of dry stored hairs with hair roots attached. Please do not fix hairs on adhesive surfaces, this hinders later analyses. Fixing hairs on adhesive strips etc. is only useful in exceptional cases (e.g. when searching areas for hair and tissue remnants, e.g. in forensic examinations).

Scats (Feces) are placed in a plastic container¹ filled with approx. 33 ml undenatured ethanol¹⁰ (96%; see figure overleaf). 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. Caution: larger quantities of sample containers may only be stored in explosion-proof refrigerators/freezers. Ideally, a sterile object (scalpel, tweezers, cleaned, flamed blade of a pocket knife; a wooden stick cut to size is also suitable; this prevents cross-contamination via an unclean blade if no suitable cleaning facilities are available) should be used to collect scats. 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. Often 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).

Tissue (ideally muscle tissue the 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 refrigerators/freezers. If no suitable sample containers or 96% ethanol are available, the tissue can also be stored in a container with other (preferably high-percentage) alcohol if necessary. The containers used 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. The container should be labelled with a pencil (avoids smudging).

Blood (depending on the amount available) should be collected with up to three sterile cotton swabs, placed in a folded filter paper⁷ and then stored in a ziplock bag³ with desiccant⁴ at room temperature (see figure overleaf). For sampling dried blood traces, it is recommended to moisten the cotton 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 immediately with cold storage. Alternatively, blood samples (up to a maximum of 3 ml) can be placed in containers with 33 ml of pure (at least 96%) ethanol¹⁰, stored at room temperature and shipped. If no collection materials are available, blood can be collected with a tissue or piece of paper and dried.

Other - Bones and teeth are placed in a ziplock bag³ with desiccant⁴ and stored at room temperature. **Saliva** (as an invasive oral mucosal swab) is preferably collected using cotton swabs¹¹. These can be stored analogously to other swab samples (tear swabs, blood) in tubes or ziplock bags provided for this purpose with filter paper and drying agent. **Urine** also contains DNA and can best be collected in winter as a urine trace 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 in compliance with the cold chain. Alternatively (e.g. in case of small amounts of urine & estrus blood), the urine can be collected with one or two swabs. To do this, let the urine drops thaw in frozen snow on the disposable glove and soak the swab(s) in it.

Special cases: If you suspect an attack on humans by a wild animal, you should always contact us before taking a sample.