Jan 14, 2026

Plasma isolation for cell-free DNA analysis from dogs using EDTA blood tubes

Cancer Research Communications

DOI
https://dx.doi.org/10.17504/protocols.io.ewov11bzyvr2/v1

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DOI: https://dx.doi.org/10.17504/protocols.io.ewov11bzyvr2/v1

External link: https://doi.org/10.1158/2767-9764.CRC-25-0373

Protocol Citation: Patricia Filippsen Favaro, Muhammed Murtaza 2026. Plasma isolation for cell-free DNA analysis from dogs using EDTA blood tubes. **protocols.io** https://dx.doi.org/10.17504/protocols.io.ewov11bzyvr2/v1

Manuscript citation:
Favaro PF, Wang Y, McDonald BR, Wang TX, Jadhav S, Cheng HH, Marcinak CT, Pan X, Murtaza M (2026) Comparative Analysis of Cell-Free DNA Fragmentation Patterns in Canines with Sarcoma and Tumor-Free Canines and Humans. Cancer Research Communications 6(2). doi: 10.1158/2767-9764.CRC-25-0373

Protocol status: Working
We use this protocol and it's working

Created: November 07, 2025

Last Modified: January 14, 2026

Protocol Integer ID: 231793

Keywords: cell-free DNA, canine, blood, plasma, liquid biopsy, free dna analysis from dog, edta blood tubes laboratory protocol for blood processing, using edta blood tubes laboratory protocol, plasma isolation for cell, tubes laboratory protocol for blood processing, free dna analysis, using blood sample, blood sample, plasma isolation, tubes laboratory protocol, edta tube, blood processing, laboratory, dna

Disclaimer

Abstract

Laboratory protocol for blood processing and plasma isolation for cell-free DNA analysis from dogs using blood samples collected in EDTA tubes.

Guidelines

1. After blood collection, turn the tube gently 10 times to homogenize the blood and place at room temperature, in upright position, until processing.
2. It is critical that blood samples are centrifuged twice as described in the protocol, within 1-3 hours of collection to minimize the possibility of white blood cells (WBCs) lysis that can affect ability to measure circulating tumor DNA (ctDNA).
3. Collect buffy coat in a separate 2 mL sterile screw-capped DNase-free microcentrifuge tube.
4. Collect plasma aliquots in separate 2 mL sterile screw-capped DNase-free microcentrifuge tubes.

Procedure

1 Collect blood in K2-EDTA tubes with 6 mL or 3 mL capacity, based on expected blood volume and size of the dog
Note: The tube should be filled during collection to ensure the correct proportion of additive and blood.

2 Gently invert the tube 10 times, and leave it on upright position at room temperature prior to centrifugation.

3 Within 1-2 hours, centrifuge the tubes at 820 x g for 10 minutes at room temperature, with the centrifuge brake off (gentle slowdown speed).

4 Collect the supernatant (plasma), transferring 1 mL aliquots to microtubes, taking care to not disturb the whitish band (which is the buffy coat).
Label the tubes: #1 #2 #3 (...) in the order of aliquoting the plasma.
Use these in Step 6.

5 Collect the buffy coat (~700 µL) in a separate microtube (cryotube, screw-capped): start collecting from slightly above (~2 mm) the white band.
Note: It is expected to pipette a small amount of red blood cells during buffy coat collection.
Store microtubes with buffy coat at -80˚C.

6 Centrifuge the plasma aliquots (from step 4) at 16,000 x g for 10 minutes at room temperature, with the centrifuge brake off (gentle slowdown speed).
Record the time that the centrifugation started (centrifugation #2).
Note: a pellet will likely be formed.

7 Collect the supernatant (plasma), transferring 900 µL of each aliquot to a new microtube (cryotube, screw-capped)
Note: be careful to not disturb the pellet.
Store microtubes at -80˚C.