Dec 18, 2025

# Quick-Start Protocol DNeasy Blood Tissue Kit and Genotyping

DOI
https://dx.doi.org/10.17504/protocols.io.8epv557onv1b/v1

Janna Jernigan Posey[1], Malu Tansey[1]

[1]Indiana University

Janna Jernigan Posey
Indiana University





**DOI:** https://dx.doi.org/10.17504/protocols.io.8epv557onv1b/v1

**Protocol Citation:** Janna Jernigan Posey, Malu Tansey 2025. Quick-Start Protocol DNeasy Blood Tissue Kit and Genotyping. **protocols.io** https://dx.doi.org/10.17504/protocols.io.8epv557onv1b/v1



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

**Created:** December 18, 2025

**Last Modified:** December 19, 2025

**Protocol Integer ID:** 235377

**Keywords:** ASAPCRN, genotyping blood dna extraction, start protocol dneasy blood, blood dna extraction, tissue kit, snp

**Funders Acknowledgements:**
**The Michael J. Fox Foundation for Parkinson's Research (MJFF) and the Aligning Science Across Parkinson's (ASAP)**
Grant ID: ASAP-020527

## Abstract

Blood DNA extraction and SNP genotyping

## Materials

DNeasy Blood 26 Tissue Kit (cat. nos. 69504 and 69506), Buffer AL, Buffer ATL, Buffer AW1, Buffer AW2, Buffer AE, Proteinase K, PBS, Ethanol (96 and;100%), Microcentrifuge tubes (1.5 ml or 2 ml), DNeasy Mini spin column, Centrifuge, Incubator, Optical Adhesive Film, Taqman probe, 1XTE Buffer, Taqman Universal PCR MM, Denovix.

## Before start

Perform all centrifugation steps at room temperature (15 and 25 degrees C). Redissolve any precipitates in Buffer AL and Buffer ATL. Add ethanol to Buffer AW1 and Buffer AW2 concentrates. Equilibrate frozen tissue or cell pellets to room temperature. Preheat an incubator to 56 degreesC. Refer to the handbook for pretreatment of fixed tissue, insect, bacterial or other material.

## DNA Extraction Protocol

1 Sample Prep

1.1 Tissue: Cut tissue (≤10 mg spleen or ≤25 mg other tissue) into small pieces, and place in a 1.5 ml microcentrifuge tube. For rodent tails, use 1 (rat) or 2 (mouse) 0.4–0.6 cm lengths of tail. Add 180 µl Buffer ATL. Add 20 µl proteinase K, mix by vortexing and incubate at 56°C until completely lysed. Vortex occasionally during incubation. Vortex 15 s directly before proceeding to step 2.

1.2 Nonnucleated blood: Pipet 20 µl proteinase K into a 1.5 ml or 2 ml microcentrifuge tube. Add 50–100 µl anticoagulant-treated blood. Adjust volume to 220 µl with PBS. Proceed to step 2.

1.3 Nucleated blood: Pipet 20 µl proteinase K into a 1.5 ml or 2 ml microcentrifuge tube. Add 5–10 µl anticoagulant-treated blood. Adjust volume to 220 µl with PBS. Proceed to step 2.

1.4 Cultured cells: Centrifuge a maximum of 5 × 10^6 cells for 5 min at 300 x g (190 rpm). Resuspend in 200 µl PBS. Add 20 µl proteinase K. Proceed to step 2.

2 Add 200 µl Buffer AL. Mix thoroughly by vortexing. Incubate blood samples at 56°C for 10 min.

3 Add 200 µl ethanol (96–100%). Mix thoroughly by vortexing.

4 Pipet the mixture into a DNeasy Mini spin column placed in a 2 ml collection tube. Centrifuge at ≥6000 x g (8000 rpm) for 1 min. Discard the flow-through and collection tube.

5 Place the spin column in a new 2 ml collection tube. Add 500 µl Buffer AW1. Centrifuge for 1 min at ≥6000 x g. Discard the flow-through and collection tube.

6 Place the spin column in a new 2 ml collection tube, add 500 µl Buffer AW2 and centrifuge for 3 min at 20,000 x g (14,000 rpm). Discard the flow-through and collection tube.

7 Transfer the spin column to a new 1.5 ml or 2 ml microcentrifuge tube.

8 Elute the DNA by adding 200 µl Buffer AE to the center of the spin column membrane. Incubate for 1 min at room temperature (15–25°C). Centrifuge for 1 min at ≥6000 x g.

9 Optional: Repeat step 7 for increased DNA yield.

10 Determine the concentration of DNA using the denovix.

11 DNA can be stored in -20°C until genotyping.

## Genotyping Protocol

12 Obtain your DNA sample and Known Allell control samples (Example: Known sample that is Homozygous for AA, known sample that is Homozygous for GG, known sample that is Heterozygous AG)

13 Dilute DNA sample to 25ng/reaction with nuclease free water with a final volume of 9ul.

14 Make taqman master mix by combining 0.5ul/sample of 40X primer and taqman probe, 0.5ul/sample of 1XTE Buffer, and 10ul/sample of Taqman Universal PCR MM (specific to the SNP you are looking at). Best practice is to make enough MM for all your samples and few more.

15 Add 11ul of taqman master mix to each diluted DNA sample.

16 Mix and reverse pipette samples + master mix into 384 well plate in triplicate.

17 Seal plate with Optical Adhesive Film.

18 Centrifuge plate at 3000rpm for 5 min at 4°C.

19 Immediately transfer plate to the Quant Studio v5 and run genotyping program that includes the following methods and temperatures.

19.1 1) Pre-read fluorescence levels
2) 10 minutes at 95°C
3) 40 cycles of 92°C for 15 seconds followed by 60°C for 1 minute
4) Post-read fluorescence levels

20 Sample concentration may need to be tested. Some starting concentrations could include 12.5ng/reaction, 25ng/reaction, and 50ng/reaction. If samples are undetermined after running the program you may need to increase sample reaction concentration.

## Acknowledgements

Qiagen DNAeasy blood and tissue kit and Taqman SNP genotyping