6. Add 5 µl of Proteinase K Solution using a new pipette tip for each sample. Vortex 5 seconds.
7. Add 10 µl of Binding Buffer to each sample. Vortex 5 seconds.
8. Incubate samples in a 54-56˚C water bath for 30 minutes. The suspension should clear by the end of the incubation unless the sample contains a very high load of cells.
9. While samples are incubating, warm a tube of sterile deionized water (100 µl per sample) to 54-56˚C for use in Step 19, below.
10. Add 150 µl of Binding Buffer to each sample, vortex 5 seconds, and spin at a minimum of 10,000 x g for 1 minute.
11. Separately, transfer the supernatant (~220 µl) to a new prelabeled Spin Column Assembly (SCA). Avoid pipetting the pelleted cell debris; only transfer the liquid portion of the specimen to the Spin Column.
12. Centrifuge each SCA at a minimum of 10,000 x g for 1 minute.
13. Add 200 µl of Wash Buffer 1 (1x) to each Spin Column and spin at a minimum of 10,000 x g for 1 minute.
14. Discard the flow-through liquid by decanting.
15. Add 500 µl of Wash Buffer 2 (1x) to each Spin Column and spin at a minimum of 10,000 x g for 1 minute.
16. Discard the flow-through liquid by decanting.
17. Spin each Spin Column Assembly at a minimum of 10,000 x g for 1 minute.
18. Detach each Spin Column from its bottom reservoir and place in a fresh pre-labeled microcentrifuge tube.
19. Carefully pipette 50 µl of pre-warmed sterile deionized water from Step 9 into the center of each Spin Column. Incubate for 2 minutes at room temperature 15-30˚C.
20. Centrifuge each Spin Column at a minimum of 10,000 x g for 1 minute. Remove Spin Column and cap lower labeled microcentrifuge tube containing the eluted DNA. Note: For a ~50% higher (though more dilute) yield, repeat steps 19-20. When finished eluting, discard the Spin column
21. Samples are now ready for DNA analysis. Purified samples can be stored at 2-8˚C for up to 1 week, or frozen (≤-20˚C) for extended storage.

REFERENCES

1. Centers for Disease Control and Prevention. Perspectives in Disease Prevention and Health Promotion Update: Universal Precautions for Prevention of Transmission of Human Immunodeficiency Virus, Hepatitis B Virus, and Other Bloodborne Pathogens in Health-Care Settings. MMWR, June 24,1988/37(24):377-388

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Asanté™ DNA

DNA Purification Kit

Cat. No. 1802

NAME AND INTENDED USE

The Asanté DNA Purification Kit is intended as an adjunct to the Asanté DNA Specimen Collection Kit (Cat. No. 1800) for the extraction and purification of DNA from specimens collected by the Asanté DNA Specimen Collection Kit. DNA from specimens collected by the Asanté DNA Specimen Collection Kit and purified using the Asanté DNA Purification Kit are suitable for testing by most in vitro DNA amplification methods including polymerase chain reaction (PCR) and modifications, pretreatments and extensions thereof, helicase-dependent amplification (HAD), ligase chain reaction (LCR), loop-mediated isothermal amplification (LAMP), next gen DNA sequencing (NGS) and others.

For Research Use Only. Not for Use in Diagnostic Procedures.

MATERIALS PROVIDED

The Asanté DNA Purification Kit is available in 3 package sizes. Materials provided for each package size are described in Table 1 below:

Table 1. Asanté DNA Purification Kit Components

Cat. No.	1802-010	1802-050	1802-250
Number of purifications per kit	10	50	250
RNAse A Solution	1 vial (100 µL)	1 vial (300 µL)	1 vial (1 mL)
Proteinase K Solution	1 vial (100 µL)	1 vial (300 µL)	1 vial (1 mL)
Binding Buffer	1 bottle (2 mL)	1 bottle (9 mL)	1 bottle (44 mL)
Wash Buffer 1, 2X	1 bottle (1.2 mL), [2.4 mL final diluted vol.]	1 bottle (6 mL), [12 mL final diluted vol.]	1 bottle (30 mL), [60 mL final diluted vol.]
Wash Buffer 2, 2X	1 bottle (3 mL), [6 mL final diluted vol.]	1 bottle (15 mL), [30 mL final diluted vol.]	1 bottle (75 mL), [150 mL final diluted vol.]
Spin Column Assemblies	10	50	250
Product Insert	1	1	1

MATERIALS REQUIRED BUT NOT PROVIDED

- Pipettes, 1000 µL, 200µL and 10µL
- Microcentrifuge tubes, 1-5-2.0 mL, polypropylene
- Microcentrifuge tube labels
- Vortex mixer
- Timer
- Microcentrifuge capable of 10,000 x g with rotor to hold 1.5-2.0 mL microcentrifuge tubes and/or Spin Column Assemblies.
- Ethanol, 95-100% v/v
- Waterbath, 54-56°C
- Sterile deionized water
- Personal protective equipment (PPE) (disposable gloves, safety glasses, etc.)
- Disinfectant as required by Universal Precautions and biohazardous waste container.

WARNINGS AND PRECAUTIONS

1. The Asanté DNA Purification Kit is for use only as an adjunct to the Asanté DNA Specimen Collection Kit. Use of the Asanté DNA Purification Kit with any other collection kit device or samples may produce erroneous results.
2. The performance characteristics of the Asanté DNA Specimen Collection Kit and Asanté DNA Purification Kit have not been established for all applications. It is recommended that studies specific for the intended application are conducted by the user and properly validated.
3. Storage of purified DNA specimens obtained from the purification procedure in the Asanté DNA Purification Kit should be at ≤ -20°C. Storage at 2-8°C should not exceed 1 week.
4. All human tissue and body fluid specimens, and materials they come in contact with, should be handled as if potentially infectious, in accordance with "Universal Precautions".[1] In the U.S., Occupational Safety and Health Administration (OSHA) regulations (29 CFR 1910) apply to personnel collecting and handling human clinical specimens. Follow any other local regulations for biohazardous agents.
5. Federal, state and local regulations for human biological test specimens apply to the mailing, transportation or shipment of specimens which may contain infectious agents.
6. Do not reuse the Spin Column Assemblies or their components.
7. The Asanté DNA Purification Kit is not designed for the purification of RNA.

IMPORTANT PROCEDURAL NOTES

1. Do not use the Asanté DNA Purification Kit or kit components beyond the expiration dates specified on the product and component labeling. Storage of Kit materials at temperatures except as specified may result in diminished assay performance and may give inaccurate results.
2. The Asanté DNA Purification Kit should be stored at 2-8°C upon receipt.
3. Wash Buffer 1 and Wash Buffer 2, after dilution with ethanol, may be stored for up to 6 months at 2-8°C. Any crystals which form should be re-dissolved with heating (≤ 37°C).
4. Kit components should be brought up to room temperature (15-30°C) before use.
5. The DNA Purification Kit procedure should be conducted at room temperature (15-30°C) except where otherwise noted.
6. Use only calibrated pipettes for all measurements. Always use separate filtered pipette tips, and tubes for each specimen. Do not interchange Kit bottles or vial caps.
7. Mix all reagents immediately before use.
8. Return all Kit components to their recommended storage conditions immediately after use.

REAGENT PREPARATION

1. Prepare working Wash Buffer 1 and Wash Buffer 2 by diluting each of these reagents as supplied in the Kit, 1:2 in ethanol (95-100% v/v). To prepare the final buffers in the bottles the 2X reagents are supplied in, add ethanol as shown in Table 2 on the right. Check the box on the label to indicate that ethanol has been added.
2. Wash Buffers diluted to 1X working dilution should be stored at 2-8°C and should be used within 6 months of dilution or before expiration of the original 2X reagent, whichever comes first.

Table 2. Preparation of Working Dilutions of Wash Buffers 1 and 2

Cat. No.	1802-010	1802-050	1802-250
Number of purifications per kit	10	50	250
Wash Buffer 1, 2X, packaged volume	1.2 mL	6 mL	30 mL
Add mL Ethanol (95-100%)	1.2 mL	6 mL	30 mL
Final Volume Wash Buffer 1, 1X	2.4 mL	12 mL	60 mL
Wash Buffer 2, 2X, packaged volume	3 mL	15 mL	75 mL
Add mL Ethanol (95-100%)	3 mL	15 mL	75 mL
Final Volume Wash Buffer 1, 1X	6 mL	30 mL	150 mL

PROCEDURE FOR DNA PURIFICATION

1. Resuspend specimen in the Asanté DNA Specimen Collection Kit Sample Buffer by inverting the capped Specimen Tube up and down several times, and then tapping the bottom of the tube to dislodge any residual liquid trapped in the cap. Using a 1000 µl pipette, transfer all of the Sample Buffer into a labeled microcentrifuge tube.
2. Cap the tube and centrifuge in the microcentrifuge at a minimum of 10,000 x g for 1 minute.
3. Decant most of the supernatant with a 1000 µl pipette, taking care not to disturb the loose cell pellet at the bottom of the microcentrifuge tube. Leaving a small amount of liquid in the tube is permissible. If the pellet is disturbed, repeat steps 2 and 3, above.
4. Add 50 µl of sterile deionized water to each sample pellet.
5. Add 5 µl of RNase A Solution, using a new pipette tip for each sample. Vortex 5 seconds. The solution may appear hazy.