Clean Blood & Tissue DNA Kit

Catalog Nos. CBT-D0096, CBT-D0384
Manual revision v3.00

Contents

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Introduction and Principle

The Clean Blood DNA Kit is based upon our proprietary magnetic beads based system to extract high quality genomic DNA. It can be used for the genomic DNA isolation from 1-200 µL of fresh or frozen whole blood, buffy coat containing anticoagulants such as Citrate, EDTA and Heparin as well as DNA isolation from saliva, tissue, buccal swabs, mouse tail snips and cultured cells.

The kits protocol is fully scalable and due to the use of our magnetic bead purification technology, can besides manual usage, easily be automated on liquid handling workstations (e.g. Beckman, Hamilton, Tecan, Caliper, Perkin Elmer, Agilent and Eppendorf). The procedure eliminates the needs for vacuum steps providing hands-free operation in automated protocols.

Our Clean Blood DNA Kit enables high quality DNA to be isolated that is suitable for direct use in most downstream applications.

The samples are lysed using our special formulated lysis buffers, which are optimized for the various types of starting material. DNA is isolated from the lysates in one step by binding to CleanNA Particles' surfaces. The CleanNA magnetic particles are separated from the lysates by using a magnetic separation device. Following a few rapid wash steps to remove trace contaminants, the purified DNA is eluted from the CleanNA particles for downstream applications using an elution buffer.

Kit Contents and Materials

Kit Contents:

Product	CBT-D0096	CBT-D0384	Storage
Preps	96	384	
BT Tissue Lysis Buffer	30 mL	120 mL	15-25^{0}C
BT Lysis Buffer	35 mL	125 mL	15-25^{0}C
BT Binding Buffer	10 mL	40 mL	15-25^{0}C
BT Wash Buffer 1	55 mL	220 mL	15-25^{0}C
BT Wash Buffer 2	30 mL	120 mL	15-25^{0}C
Proteinase K Solution (20 mg/ml)	2.2 mL	9 mL	2-8^{0}C
Elution Buffer	50 mL	200 mL	15-25^{0}C
CleanNA Particles CBT	2.2 mL	9 mL	2-8^{0}C

Materials and Reagents to be supplied by User for <u>Blood</u> and <u>Tissue</u> protocol:

- 100% ethanol
- 100% isopropanol (IPA)
- Magnetic separation device for 96-well plates from CleanNA (Cat# CMAG-RN50)
- Centrifuge with swing-bucket rotor capable of 4,000 x g
- Centrifuge adaptor for 96-well plates
- Shaking water bath capable of 55°C
- 96-well Round-well Plate (Recommended ABgene, Part#AB-0661 (2,2 mL)
- 96-well plate for storage of isolated gDNA
- Vortexer
- Multichannel pipettes and reagent reservoirs
- Sealing film
- Optional: Heat block, incubator, or water bath capable of 70°C
- Optional: PBS or nuclease-free water
- Optional: RNase A (10 mg/mL)

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Materials and Reagents to be supplied by User for Cultured Cells protocol:

- 100% ethanol
- 100% isopropanol
- Nuclease-free water
- Magnetic separation device for 96-well plates from CleanNA (Cat# CMAG-RN50)
- Vortexer
- Shaking water bath capable of 55°C
- 96-well plate for storage of isolated gDNA
- 2 mL 96-well deep-well plates (Recommend Nunc, Part#278752 or ABgene, Part#AB-0661) or desired plate compatible with the magnetic separation device
- Multichannel pipettes and reagent reservoirs
- Sealing film
- Cold PBS (4°C)
- Optional: RNase A (10 mg/mL)
- Optional: Heat block, incubator, or water bath capable of 70°C

Materials and Reagents to be supplied by User for Saliva protocol:

- 100% ethanol
- 100% isopropanol
- Nuclease-free water
- Magnetic separation device for 96-well plates from CleanNA (Cat# CMAG-RN50)
- Vortexer
- 96-well plate for storage of isolated gDNA
- 2 mL 96-well deep-well plates (Recommend Nunc, Part#278752 or ABgene, Part#AB-0661) or desired plate compatible with the magnetic separation device
- Multichannel pipettes and reagent reservoirs
- Sealing film
- Optional: RNase A (10 mg/mL)
- Optional: Heat block, incubator, or water bath capable of 70°C

Materials and Reagents to be supplied by User for Buccal Swabs and Mouse Tail Snips protocol:

- 100% ethanol
- 100% isopropanol
- Nuclease-free water
- Magnetic separation device for 96-well plates from CleanNA (Cat# CMAG-RN50)
- Vortexer
- Centrifuge with swing-bucket rotor capable of 4,000 x g
- Centrifuge adaptor for 96-well plates
- Shaking water bath capable of 55°C
- 96-well plate for storage of isolated gDNA
- 2 mL 96-well deep-well plates (Recommend Nunc, Part#278752 or ABgene, Part#AB-0661) or desired plate compatible with the magnetic separation device
- Multichannel pipettes and reagent reservoirs
- Sealing film
- Optional: RNase A (10 mg/mL)
- Optional: Heat block, incubator, or water bath capable of 70°C
- Optional: Clean Collector Kit (CCOL-S0100; CCOL-C1000; CCOL-A0010)

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Preparation of Reagents

Prepare BT Binding Buffer with 100% isopropanol (IPA) as follows and store at room temperature.

Kit	100% Isopropanol to be Added
CBT-D0096	40 mL
CBT-D0384	160 mL

Prepare BT Wash Buffer 1 with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
CBT-D0096	70 mL
CBT-D0384	280 mL

Dilute BT Wash Buffer 2 with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
CBT-D0096	70 mL
CBT-D0384	280 mL

Shake or vortex the CleanNA Particles CBT to fully resuspend the particles prior to usage. The particles must be fully suspended during use to ensure proper binding.

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Clean Blood & Tissue DNA Kit
96 Protocol for up to 250 µL Blood

The procedure below has been optimized for use with 250 µL FRESH or FROZEN blood samples. Buffy coat can also be used.

Before Starting:

- Prepare all Reagents according to Preparing Reagents section on Page 4.

Protocol:

1. Add blood samples to a 96-well Round-well Plate (2.2 mL). Bring the volume up to 250 µL with PBS (not provided) or Elution Buffer (provided with this kit) if volume of blood is less than 250 µL.

2. Add 20 µL Proteinase K Solution to each sample. Vortex or pipet up and down 20 times to mix.

   Optional: Add 5 µL RNase A to each sample. Vortex or pipet up and down 20 times to mix.

3. Add 290 µL BT Lysis Buffer to each sample. Vortex at maximum speed or pipet up and down 20 times.

   

   Note: This step is critical for good yields. BT Lysis Buffer must be thoroughly mixed. For automation, tip mixing is preferable to orbital shakers.

4. Incubate the plate for 10 minutes at room temperature.

   

   Note: Optional, to improve yield, the 10 minutes incubation can be performed at 70 C.

5. Add 400 µL BT Binding Buffer and 20 µL CleanNA Particles CBT to each sample.

6. Vortex at maximum speed for 10 minutes.

7. Place the plate on a magnetic separation device to magnetize the CleanNA Particles CBT. Incubate at room temperature until the CleanNA Particles CBT are completely cleared from solution.

8. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CBT.

9. Remove the plate containing the CleanNA Particles CBT from the magnetic separation device.

10. Add 600 µL BT Wash Buffer 1 to each sample.

    

    Note: BT Wash Buffer 1 must be diluted with ethanol prior to use. Please see Page 4 for instructions.

11. Resuspend the CleanNA Particles CBT by pipetting up and down 20 times or vortexing for 1 minute.

    

    Note: Complete resuspension of the CleanNA Particles CBT is critical for obtaining good purity.

12. Place the plate on the magnetic separation device to magnetize the CleanNA Particles CBT. Incubate at room temperature until the CleanNA Particles CBT are completely cleared from solution.

13. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CBT.

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14. Remove the plate containing the CleanNA Particles CBT from the magnetic separation device.

15. Repeat Steps 10-14 for a second BT Wash Buffer 1 wash step.

16. Add 600 µL BT Wash Buffer 2 to each sample.

Note: BT Wash Buffer 2 must be diluted with ethanol prior to use. Please see Page 4 for instructions.

17. Resuspend the CleanNA Particles CBT by pipetting up and down 20 times or vortexing for 1 minute.

18. Incubate at room temperature for 1 minute.

19. Place the plate on the magnetic separation device to magnetize the CleanNA Particles CBT. Incubate at room temperature until the CleanNA Particles CBT are completely cleared from solution.

20. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CBT.

21. Leave the plate on the magnetic separation device. Add 200 µL nuclease free water to the samples. Immediately aspirate liquid within 60 seconds.

Do not leave nuclease-free water on CleanNA Particles CBT for more than 60 seconds.

22. Remove the plate containing the CleanNA Particles CBT from the magnetic separation device.

23. Add 50-200 µL Elution Buffer or nuclease-free water to elute DNA from the CleanNA Particles CBT. Resuspend the CleanNA Particles CBT by pipetting up and down 50 times. .

Note: Optional, to improve yield, heat the elution buffer or nuclease-free water to 70 C.

24. Incubate at room temperature for 10 minutes.

25. Place the plate on the magnetic separation device to magnetize the CleanNA Particles CBT. Incubate at room temperature until the CleanNA Particles CBT are completely cleared from solution.

26. Transfer the cleared supernatant containing purified DNA to a clean microplate (not supplied). Store the DNA at -20°C.

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Clean Blood & Tissue DNA Kit
Protocol for Tissue Samples (up to 10 mg)

This protocol has been optimized for use with 10 mg mouse tail samples.

Before Starting:

- Prepare all Reagents according to Preparing Reagents section on Page 4.
- Set a shaking water bath to 56°C

Protocol:

1. Mince up to 10 mg of tissue or two pieces of mouse tail (0.2 - 0.5 cm in length) into a 96-well deep-well plate.

2. Add 250 µL BT Tissue Lysis Buffer.

   

   Note: Cut the tissue into small pieces to speed up lysis.

3. Add 20 µL Proteinase K Solution. Seal the plate with sealing film. Vortex to mix thoroughly.

4. Incubate at 56°C for 1-3 hours in a shaking water bath.

   

   Note: If a shaking water bath is not available, incubate the plate in an incubator and vortex the plate every 20-30 minutes. Lysis time depends on the amount and type of tissue but is usually less than 3 hours. Lysis can proceed overnight.

   Optional: Add 5 µL RNase A to each sample. Vortex or pipet up and down 20 times to mix. Incubate at room temperature for 2 minutes.

5. Centrifuge at 3,000 x g for 5 minutes at room temperature.

6. Transfer 200 µL cleared lysate into a new 96 deep-well plate.

7. Add 230 µL BT Lysis Buffer to each sample. Vortex at maximum speed for 10 minutes.

   

   Note: This step is critical for good yields. BT Lysis Buffer must be thoroughly mixed. For automation, tip mixing is preferrable to orbital shakers.

8. Add 320 µL BT Binding Buffer and 20 µL CleanNA Particles CBT to each sample. Vortex at maximum speed for 10 minutes.

9. Place the plate on a magnetic separation device to magnetize the CleanNA Particles CBT. Incubate at room temperature until the CleanNA Particles CBT are completely cleared from solution.

10. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CBT.

11. Remove the plate containing the CleanNA Particles CBT from the magnetic separation device.

12. Add 600 µL BT Wash Buffer 1 to each sample.

    

    Note: BT Wash Buffer 1 must be diluted with ethanol prior to use. Please see Page 4 for instructions.

13. Resuspend the CleanNA Particles CBT by pipetting up and down 20 times or vortexing for 1 minute.

    

    Note: Complete resuspension of the CleanNA Particles CBT is critical for obtaining good purity.

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14. Place the plate on the magnetic separation device to magnetize the CleanNA Particles CBT. Incubate at room temperature until the CleanNA Particles CBT are completely cleared from solution.

15. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CBT.

16. Remove the plate containing the CleanNA Particles CBT from the magnetic separation device.

17. Repeat Steps 12-16 once for a second BT Wash Buffer 1 wash step.

18. Add 600 µL BT Wash Buffer 2 to each sample.

Note: BT Wash Buffer 2 must be diluted with ethanol prior to use. Please see Page 4 for instructions.

19. Resuspend the CleanNA Particles CBT by pipetting up and down 20 times or vortexing for 1 minute.

20. Incubate at room temperature for 1 minute.

21. Place the plate on the magnetic separation device to magnetize the CleanNA Particles CBT. Incubate at room temperature until the CleanNA Particles CBT are completely cleared from solution.

22. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CBT.

23. Leave the plate on the magnetic separation device. Add 200 µL nuclease free water to the samples. Immediately aspirate liquid within 60 seconds.

Do not leave nuclease-free water on CleanNA Particles CBT for more than 60 seconds.

24. Remove the plate containing the CleanNA Particles CBT from the magnetic separation device.

25. Add 100-200 µL Elution Buffer or nuclease-free water to elute DNA from the CleanNA Particles CBT. Resuspend the CleanNA Particles CBT by pipetting up and down 50 times.

26. Incubate at room temperature for 5-10 minutes.

Note: heating elution buffer to 60°C prior to adding or incubating at 70°C can increase yield.

27. Place the plate on the magnetic separation device to magnetize the CleanNA Particles CBT. Incubate at room temperature until the CleanNA Particles CBT are completely cleared from solution.

28. Transfer the cleared supernatant containing purified DNA to a clean microplate (not supplied). Store the DNA at -20°C.

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Clean Blood & Tissue DNA Kit
Protocol for Buccal Swabs

Before Starting:

- Prepare all Reagents according to Preparing Reagents section on Page 4.
- Set shaking water bath to 55°C.

Protocol:

Optional: Step 1 till 5 can be performed using the CleanNA Clean Collector Kit. See the "Clean Collector User Manual" for instructions.

1. Cut off the buccal brush or swab head and place each swab into a well of a 96-well deep-well plate.

2. Add 290 µL BT Lysis Buffer and 250 µL Elution Buffer.

   Note: BT Lysis Buffer and Elution Buffer can be prepared as a mastermix.

3. Add 20 µL Proteinase K Solution.

4. Incubate at 55°C in a shaking water bath for 10 minutes.

   Note: If a shaking water bath is not available, vortex the plate every 5-10 minutes.

5. Centrifuge at 3000 x g for 2 minutes.

6. Transfer 500 µL lysate into a new 96-well deep-well plate. Do not transfer the swabs to the new plate.

   Optional: Add 5 µL RNase A to each sample. Vortex or pipet up and down 20 times to mix.

7. Add 350 µL BT Binding Buffer and 20 µL CleanNA Particles CBT to each sample. Vortex at maximum speed for 10 minutes.

   Note: BT Binding Buffer must be diluted with isopropanol prior to use. Please see Page 4 for instructions. BT Binding Buffer and CleanNA Particles CBT can be prepared as a mastermix. Mix only what is needed.

8. Place the plate on a magnetic separation device to magnetize the CleanNA Particles CBT. Incubate at room temperature until the CleanNA Particles CBT are completely cleared from solution.

9. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CBT.

10. Remove the plate containing the CleanNA Particles CBT from the magnetic separation device.

11. Add 600 µL BT Wash Buffer 1 to each sample.

    Note: BT Wash Buffer 1 must be diluted with ethanol prior to use. Please see Page 4 for instructions.

12. Resuspend the CleanNA Particles CBT by pipetting up and down 20 times or vortexing for 1 minute.

    Note: Complete resuspension of the CleanNA Particles CBT is critical for obtaining good purity.

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13. Place the plate on the magnetic separation device to magnetize the CleanNA Particles CBT. Incubate at room temperature until the CleanNA Particles CBT are completely cleared from solution.

14. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CBT.

15. Remove the plate containing the CleanNA Particles CBT from the magnetic separation device.

16. Repeat Steps 11-15 for a second BT Wash Buffer 1 wash step.

17. Add 600 µL BT Wash Buffer 2 to each sample.

Note: BT Wash Buffer 2 must be diluted with ethanol prior to use. Please see Page 4 for instructions.

18. Resuspend the CleanNA Particles CBT by pipetting up and down 20 times or vortexing for 1 minute.

19. Incubate at room temperature for 1 minute.

20. Place the plate on the magnetic separation device to magnetize the CleanNA Particles CBT. Incubate at room temperature until the CleanNA Particles CBT are completely cleared from solution.

21. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CBT.

22. Leave the plate on the magnetic separation device. Add 500 µL nuclease-free water and immediately aspirate.

Do not leave nuclease-free water on CleanNA Particles CBT for more than 60 seconds.

23. Remove the plate containing the CleanNA Particles CBT from the magnetic separation device.

24. Add 100-200 µL Elution Buffer or nuclease-free water to elute DNA from the CleanNA Particles CBT. Resuspend the CleanNA Particles CBT by pipetting up and down 50 times.

Note: Heat Elution Buffer or nuclease-free water to 70°C to improve yield.

25. Incubate at room temperature for 5 minutes.

26. Place the plate on the magnetic separation device to magnetize the CleanNA Particles CBT. Incubate at room temperature until the CleanNA Particles CBT are completely cleared from solution.

27. Transfer the cleared supernatant containing purified DNA to a 96-well microplate (not provided). Store DNA at -20°C.

Clean Blood & Tissue DNA Kit
Protocol for Saliva

Before Starting:

- Prepare all Reagents according to Preparing Reagents section on Page 4.
- Set shaking water bath to 55°C.

Protocol:

1. Spin the Saliva tubes at 2.000 x g for 5 minutes to pellet any food particles or solid debri from the sample.

2. Transfer 250 µLstabilized saliva samples (e.g. DNA Genotek Oragene®, Mawi iSWAB™, Biomatrica® DNAgard® Saliva) into a 96-well deep-well plate.

3. Add 20 µL Proteinase K Solution.

   Optional: Add 5 µL RNase A to each sample. Vortex or pipet up and down 20 times to mix.

4. Add 290 µL BT Lysis Buffer to each sample. Vortex at maximum speed for 10 minutes or pipet up and down 20 times. Proper mixing is crucial for good yield.

   Note: Tip mixing is recommended for automated protocols.

5. Add 400 µL BT Binding Buffer and 20 µL CleanNA Particles CBT to each sample. Vortex at maximum speed for 10 minutes.

   Note: BT Binding Buffer must be diluted with isopropanol prior to use. Please see Page 4 for instructions. BT Binding Buffer and CleanNA Particles CBT can be prepared as a mastermix. Mix only what is needed.

6. Place the plate on a magnetic separation device to magnetize the CleanNA Particles CBT. Incubate at room temperature until the CleanNA Particles CBT are completely cleared from solution.

7. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CBT.

8. Remove the plate containing the CleanNA Particles CBT from the magnetic separation device.

9. Add 600 µL BT Wash Buffer 1 to each sample.

   Note: BT Wash Buffer 1 must be diluted with ethanol prior to use. Please see Page 4 for instructions.

10. Resuspend the CleanNA Particles CBT by pipetting up and down 20 times or vortexing for 1 minute.

    Note: Complete resuspension of the CleanNA Particles CBT is critical for obtaining good purity.

11. Place the plate on the magnetic separation device to magnetize the CleanNA Particles CBT. Incubate at room temperature until the CleanNA Particles CBT are completely cleared from solution.

12. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CBT.

13. Remove the plate containing the CleanNA Particles CBT from the magnetic separation device.

14. Repeat Steps 8-12 for a second BT Wash Buffer 1 wash step.

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15. Add 600 µL BT Wash Buffer 2 to each sample.

Note: Complete resuspension of the CleanNA Particles CBT is critical for obtaining good purity.

16. Resuspend the CleanNA Particles CBT by pipetting up and down 20 times or vortexing for 1 minute.

17. Incubate at room temperature for 1 minute.

18. Place the plate on the magnetic separation device to magnetize the CleanNA Particles CBT. Incubate at room temperature until the CleanNA Particles CBT are completely cleared from solution.

19. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CBT.

20. Leave the plate on the magnetic separation device. Add 500 µL nuclease-free water and immediately aspirate.

Do not leave nuclease-free water on CleanNA Particles CBT for more than 60 seconds.

21. Remove the plate containing the CleanNA Particles CBT from the magnetic separation device.

22. Add 100-200 µL Elution Buffer or nuclease-free water to elute DNA from the CleanNA Particles CBT. Resuspend the CleanNA Particles CBT by pipetting up and down 50 times.

Note: Heat Elution Buffer or nuclease-free water to 70°C to improve yield.

23. Incubate at room temperature for 5 minutes.

24. Place the plate on the magnetic separation device to magnetize the CleanNA Particles CBT. Incubate at room temperature until the CleanNA Particles CBT are completely cleared from solution.

25. Transfer the cleared supernatant containing purified DNA to a 96-well microplate (not provided). Store DNA at -20°C.

Clean Blood & Tissue DNA Kit
Protocol for Mouse Tail Snips

Before Starting:

- Prepare all Reagents according to Preparing Reagents section on Page 4.
- Set shaking water bath to 55°C.

Protocol:

1. Snip a 2-5 mm piece of mouse tail, cut into several pieces, and transfer the pieces to a 96-well deep-well plate.

   Note: Follow all regulations regarding the safe and humane treatment of animals. Mice should not be older than 6 weeks since lysis will be more difficult in older animals resulting in suboptimal DNA yields. If possible, obtain tail biopsies at 2-4 weeks and freeze samples at -70°C until DNA is extracted.

2. Add 250 µL BT Tissue Lysis Buffer.

3. Add 20 µL of Proteinase K Solution. Vortex to mix thoroughly.

4. Incubate at 55°C in a shaking water bath for 10 minutes for 1-4 hours or until lysis is complete.

   Note: If a shaking water bath is not available, vortex the samples vigorously every 20- 30 minutes. Incomplete lysis may significantly reduce DNA yields. Incubation time for complete tail lysis is dependent on length of tail snip and age of animal, e.g. a 5 mm tail piece from a 2 week old mouse typically will lyse in 2 hours. For older animals, an overnight incubation may improve yields. Note that bone and hair will not lyse.

5. Centrifuge at maximum speed for 5 minutes to pellet undigested tissue debris and hair.

6. Carefully transfer 200 µL of the supernatant to a new 96-well deep-well plate without disturbing the undigested pellet.

   Optional: Mouse tail tissue contains RNA that can purify with the DNA. This will not interfere with PCR reactions, but other enzymatic reactions may be affected. To remove RNA, add 5 µL RNase A and Incubate at room temperature for 2 minutes.

7. Add 230 µL BT Lysis Buffer to each sample. Vortex at maximum speed for 10 minutes. or pipet up and down 10 times. Proper mixing is crucial for good yield.

   Note: Tip mixing is recommended for automated protocols.

8. Add 320 µL BT Binding Buffer and 20 µL CleanNA Particles CBT to each sample. Vortex at maximum speed for 10 minutes.

   Note: BT Binding Buffer must be diluted with isopropanol prior to use. Please see Page 4 for instructions. BT Binding Buffer and CleanNA Particles CBT can be prepared as a mastermix. Mix only what is needed.

9. Place the plate on a magnetic separation device to magnetize the CleanNA Particles CBT. Incubate at room temperature until the CleanNA Particles CBT are completely cleared from solution.

10. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CBT.

11. Remove the plate containing the CleanNA Particles CBT from the magnetic separation device.

12. Add 600 µL BT Wash Buffer 1 to each sample.

    Note: BT Wash Buffer 1 must be diluted with ethanol prior to use. Please see Page 4 for instructions.

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13. Resuspend the CleanNA Particles CBT by pipetting up and down 20 times or vortexing for 1 minute.

    Note: Complete resuspension of the CleanNA Particles CBT is critical for obtaining good purity.

14. Place the plate on the magnetic separation device to magnetize the CleanNA Particles CBT. Incubate at room temperature until the CleanNA Particles CBT are completely cleared from solution.

15. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CBT.

16. Remove the plate containing the CleanNA Particles CBT from the magnetic separation device.

17. Repeat Steps 12-16 for a second BT Wash Buffer 1 wash step.

18. Add 600 µL BT Wash Buffer 2 to each sample.

    Note: BT Wash Buffer 2 must be diluted with ethanol prior to use. Please see Page 4 for instructions.

19. Resuspend the CleanNA Particles CBT by pipetting up and down 20 times or vortexing for 1 minute.

20. Incubate at room temperature for 1 minute.

21. Place the plate on the magnetic separation device to magnetize the CleanNA Particles CBT. Incubate at room temperature until the CleanNA Particles CBT are completely cleared from solution.

22. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CBT.

23. Leave the plate on the magnetic separation device. Add 500 µL nuclease-free water and immediately aspirate.

    Do not leave nuclease-free water on CleanNA Particles CBT for more than 60 seconds.

24. Remove the plate containing the CleanNA Particles CBT from the magnetic separation device.

25. Add 100-200 µL Elution Buffer or nuclease-free water to elute DNA from the CleanNA Particles CBT. Resuspend the CleanNA Particles CBT by pipetting up and down 50 times.

    Note: Heat Elution Buffer or nuclease-free water to 70°C to improve yield

26. Incubate at room temperature for 5 minutes.

27. Place the plate on the magnetic separation device to magnetize the CleanNA Particles CBT. Incubate at room temperature until the CleanNA Particles CBT are completely cleared from solution.

28. Transfer the cleared supernatant containing purified DNA to a 96-well microplate (not provided). Store DNA at -20°C.

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Clean Blood & Tissue DNA Kit
Protocol for Cultured Cells (up to 5 x 10^6 cells)

This protocol is designed for rapid isolation of up to 25 µg genomic DNA from up to 5 x 10^6 cultured cells.

Before Starting:

- Prepare all Reagents according to Preparing Reagents section on Page 4.
- Set shaking water bath to 55°C.

Protocol:

1. Prepare the cell suspension.

   a. Frozen cell samples should be thawed before starting this protocol. Pellet cells by centrifugation. Wash the cells with cold PBS (4°C) and resuspend cells in 180 µL cold PBS. Proceed with Step 2 of this protocol.

   b. For cells grown in suspension, pellet 5 x 106 cells at 1,200 x g in a centrifuge tube. Discard the supernatant, wash the cells once with cold PBS (4°C), and resuspend cells in 180 µL cold PBS. Proceed with Step 2 of this protocol.

   c. For cells grown in a monolayer, harvest the cells by either using a trypsin treatment or cell scraper. Wash cells twice in cold PBS (4°C) and resuspend the cells with 180 µL cold PBS. Proceed with Step 2 of this protocol.

2. Add 20 µL Proteinase K Solution. Immediately proceed to the next step.

3. Add 230 µL BT Lysis Buffer to each sample. Vortex at maximum speed for 10 minutes. Proper mixing is crucial for good yield.

   Note: For automated protocols tip mix yields best results and is recommended.

4. Incubate at 55°C in a shaking water bath for 10 minutes.

   Note: If a shaking water bath is not available, vortex the sample every 2-3 minutes.

5. Transfer the samples into a 96-well deep-well plate.

   Optional: Add 5 µL RNase A to each sample. Vortex or pipet up and down 20 times to mix.

6. Add 320 µL BT Binding Buffer and 20 µL CleanNA Particles CBT to each sample.

7. Vortex at maximum speed for 10 minutes.

   Note: BT Binding Buffer must be diluted with isopropanol prior to use. Please see Page 4 for instructions. BT Binding Buffer and CleanNA Particles CBT can be prepared as a mastermix. Mix only what is needed.

8. Place the plate on a magnetic separation device to magnetize the CleanNA Particles CBT. Incubate at room temperature until the CleanNA Particles CBT are completely cleared from solution.

9. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CBT.

10. Remove the plate containing the CleanNA Particles CBT from the magnetic separation device.

11. Add 600 µL BT Wash Buffer 1 to each sample.

    Note: BT Wash Buffer 1 must be diluted with ethanol prior to use. Please see Page 4 for instructions.

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12. Resuspend the CleanNA Particles CBT by pipetting up and down 20 times or vortexing for 1 minute.

    Note: Complete resuspension of the CleanNA Particles CBT is critical for obtaining good purity.

13. Place the plate on the magnetic separation device to magnetize the CleanNA Particles CBT. Incubate at room temperature until the CleanNA Particles CBT are completely cleared from solution.

14. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CBT.

15. Remove the plate containing the CleanNA Particles CBT from the magnetic separation device.

16. Repeat Steps 11-15 for a second BT Wash Buffer 1 wash step.

17. Add 600 µL BT Wash Buffer 2 to each sample.

    Note: BT Wash Buffer 2 must be diluted with ethanol prior to use. Please see Page 4 for instructions.

18. Resuspend the CleanNA Particles CBT by pipetting up and down 20 times or vortexing for 1 minute.

19. Incubate at room temperature for 1 minute.

20. Place the plate on the magnetic separation device to magnetize the CleanNA Particles CBT. Incubate at room temperature until the CleanNA Particles CBT are completely cleared from solution.

21. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CBT.

22. Leave the plate on the magnetic separation device. Add 500 µL nuclease-free water and immediately aspirate.

    Do not leave nuclease-free water on CleanNA Particles CBT for more than 60 seconds.

23. Remove the plate containing the CleanNA Particles CBT from the magnetic separation device.

24. Add 100-200 µL Elution Buffer or nuclease-free water to elute DNA from the CleanNA Particles CBT. Resuspend the CleanNA Particles CBT by pipetting up and down 50 times.

    Note: Heat Elution Buffer or nuclease-free water to 70°C to improve yield.

25. Incubate at room temperature for 5 minutes.

26. Place the plate on the magnetic separation device to magnetize the CleanNA Particles CBT. Incubate at room temperature until the CleanNA Particles CBT are completely cleared from solution.

27. Transfer the cleared supernatant containing purified DNA to a 96-well microplate (not provided). Store DNA at -20°C.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact your local distributor.

Possible Problems and Suggestions

Problem	Cause	Solution
Low DNA yield	Incomplete resuspension of CleanNA Particles CBT	Resuspend the CleanNA Particles CBT by vortexing vigorously before use
	Loss of CleanNA Particles CBT during operation	Avoid disturbing the CleanNA Particles CBT during aspiration
	DNA remains bound to CleanNA Particles CBT	Increase elution volume and Incubate at for 15 minutes; pipet up and down 50 to 100 times
	DNA washed off	Dilute BT Wash Buffer 2 by adding appropriate volume of ethanol prior to use (see Page 4 for instructions)
	Ethanol is not added into BT Wash Buffer 1	Make sure to add ethanol to the BT Wash Buffer 1 (see Page 4 for instructions)
	Frozen blood samples not mixed properly after thawing	Thaw the frozen blood at room temperature and gently mix the blood by inverting
	Inefficient cell lysis due to decrease of activity of the Proteinase K	Add more Proteinase K Solution
	Inefficient cell lysis due to inefficient mix of Lysis Buffer and Sample	Ensure the sample is thoroughly mixed with Lysis Buffer (s).
CleanNA particles CBT do not completely clear from solution	Too short of magnetizing time	Increase collection time on the magnet
Gel-like material in the eluted DNA	Blood is too old	Use 8 mM NaOH as elution buffer
		Remove the gel-like material by centrifugation; recommend using fresh blood
Problems in downstream applications	Salt carryover	BT Wash Buffer 2 must be at room temperature
	Ethanol carryover	Dry the CleanNA Particles CBT at 37°C for 5 minutes before elution

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Ordering Information

Contact your local distributor to order.

Product	Part Number
Clean Blood & Tissue DNA Kit (96 Preps)	CBT-D0096
Clean Blood & Tissue DNA Kit (4 x 96 Preps)	CBT-D0384

Product	Part Number
Clean Magnet Plate 96-well RN50	CMAG-RN50

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Notes

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