Clean Blood & Tissue DNA Kit

USER MANUAL

Manual revision v6.00

Catalog Numbers:
CBT-D0096: 96 preps
CBT-D0384: 384 preps

Batch No: See package

Shipping: Room temperature

Storage and stability: CleanNA Particles CBT should be stored at 4°C upon receipt, store all other components at room temperature. See page 3 for more storage information.

Intended use: Clean Blood & Tissue Kit is intended for use by professional users trained in molecular biology techniques. It is designed to use manually or on a liquid handling workstation for molecular biology applications.

Quality Control: Each lot of Clean Blood & Tissue is tested against predetermined specifications to ensure consistent product quality. If in any case inconsistencies occur, please contact us at info@cleanna.com or +31 (0) 182 22 33 50.

Safety precautions: When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles. Please refer to the material safety data sheet for further information.

Emergency: In case of a medical emergency due to the use of this product, contact your local poison control center. When a severe incident occurs, please inform CleanNA at +31 (0) 182 22 33 50 or info@cleanna.com.

Expiry: When stored under the recommended conditions and handled correctly, full activity is retained until the expiry date on the outer box label.

FOR RESEARCH USE ONLY

Contents

Introduction and Principle

The Clean Blood & Tissue DNA Kit is based upon our proprietary magnetic particle-based system to extract high quality genomic DNA from Blood and Tissue. It can be used for DNA isolation from 1-250 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 isolation protocol is fully scalable and, due to the use of our magnetic particle purification technology, can easily be automated on liquid handling workstations (e.g. Dynamic Devices LYNX™, Hamilton STAR™). Alternatively, each protocol can be performed manually using 96 well plates or 2 mL tubes. The isolated DNA is suitable for direct use in most downstream applications, such as next generation sequencing, PCR amplification, enzymatic reactions, etc.

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 the surface of the CleanNA Particles CBT. 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 or molecular biology grade water.

Kit Contents and Materials

Kit Contents:

Product	CBT-D0096	CBT-D0384	Storage
Preps	1 x 96	4 x 96	n/a
BT Tissue Lysis Buffer	30 mL	120 mL	15-25°C
BT Lysis Buffer	35 mL	125 mL	15-25°C
BT Binding Buffer	10 mL	40 mL	15-25°C
BT Wash Buffer 1	55 mL	220 mL	15-25°C
Proteinase K Solution (20 mg/ml)	2.2 mL	9 mL	15-25°C (for storage > 12 months, store at 2-8°C)
BT Wash Buffer 2	30 mL	120 mL	15-25°C
Elution Buffer	50 mL	200 mL	15-25°C
CleanNA Particles CBT	2.2 mL	9 mL	2-8°C

General Materials and Reagents to be supplied by user:

- 100% ethanol
- 100% isopropanol (IPA)
- Magnetic separation device for 96-well plates from CleanNA (Cat# CMAG-RN50)
- Vortexer
- 96-well plate or single tubes 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

Materials and Reagents to be supplied by user for Blood and Tissue protocol:

- Centrifuge with swing-bucket rotor capable of 4,000 x g
- Centrifuge adaptor for 96-well plates
- Shaking water bath capable to be set at 55°C
- Optional: PBS or molecular biology grade water
- Optional: DTT
- Optional: RNase A (10 mg/mL)
- Optional: Heat block, incubator, or water bath capable to be set at 70°C

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

- Molecular biology grade water
- Shaking water bath capable to be set at 55°C
- Cold PBS (4°C)
- Optional: RNase A (10 mg/mL)
- Optional: Heat block, incubator, or water bath capable to be set at 70°C

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

- Molecular biology grade water
- Optional: RNase A (10 mg/mL)
- Optional: Heat block, incubator, or water bath capable to be set at 70°C

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

- Molecular biology grade water
- Centrifuge with swing-bucket rotor capable of 4,000 x g
- Centrifuge adaptor for 96-well plates
- Shaking water bath capable to be set at 55°C
- Optional: DTT
- Optional: RNase A (10 mg/mL)
- Optional: Heat block, incubator, or water bath capable to be set at 70°C

Preparation of Reagents

BT Binding Buffer
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

BT Wash Buffer 1
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

BT Wash Buffer 2
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.

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. This protocol describes the usage of 96-well round bottom plates, but alternatively single 2 mL tubes with a compatible magnet stand can also be used.

Before Starting:

- Prepare all reagents according to the "Preparation of Reagents" section on Page 5.
- Optionally, set a heat block, incubator or water bath to 70˚C.

Protocol:

1. Add blood samples to a 96-well Round-well Plate (2.2 mL). If the volume of the bloodsample is less than 250 µL, fill to 250 µL with PBS (not provided) or Elution Buffer (provided with the kit).
2. Add 20 µL Proteinase K Solution 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.

Optional: Blood 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, mix by vortexing or pipetting up and down 20 times. Incubate at room temperature for 2 minutes.

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

   Note: BT Binding Buffer 1 must be diluted with isopropanol prior to use. Please see Page 5 for instructions.
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 5 for instructions.
11. Resuspend the CleanNA Particles CBT by pipetting up and down 15 times or vortexing for 15 seconds.

    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.

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 5 for instructions.
17. Resuspend the CleanNA Particles CBT by pipetting up and down 15 times or vortexing for 15 seconds.

    Note: Complete resuspension of the CleanNA Particles CBT is critical for obtaining good purity.
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 and select either of the following ethanol removal steps
    - **a.** Leave the plate on the magnetic separation device and add 500 µL molecular biology grade water (not provided) to the samples. Leave on the magnet for 20-30 second and the aspirate to remove the water from the CleanNA particles CBT.
      Do not leave molecular biology grade water on CleanNA Particles CBT for more than 60 seconds.
      Continue to step 22.

      OR

    - b. Leave the plate on the magnetic separation device and wait for 1 minute. Remove any residual liquid from the wells using a pipet. While leaving the plate on the magnet, dry the CleanNA Particles CBT for 10 minutes at room temperature.
      Continue to step 22.
22. Remove the plate containing the CleanNA Particles CBT from the magnetic separation device.
23. Add 50-200 µL Elution Buffer or molecular biology grade water to elute DNA from the CleanNA Particles CBT.

    Note: Optional, to improve yield, heat the elution buffer or molecular biology grade water to 70°C.
24. Resuspend the CleanNA Particles CBT by pipetting up and down 30 times or vortexing for 5 minutes.
25. Incubate at room temperature for 10 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 clean microplate or tube (not supplied). Store the DNA at -20°C.

Protocol for Tissue Samples (up to 10 mg)

This protocol has been optimized for use with up to 10 mg tissue. The protocol describes the usage of 96-well round bottom plates, but alternatively single 2 mL tubes with a compatible magnet stand can be used.

Before Starting:

- Prepare all Reagents according to Preparing Reagents section on Page 5.
- Set a shaking water bath to 56°C.
- Optionally, set a heat block, incubator or water bath to 70˚C.

Protocol:

1. Mince up to 10 mg of tissue into a 96-well deep-well plate or tube.
2. Add 250 µL BT Tissue Lysis Buffer.

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

   Optional: For lysis of tough-to-lyse tissues, such as hair, a master mix of BT Tissue Lysis Buffer and DTT (not provided) is recommended. Create a BT Tissue Lysis Buffer/DTT master mix as follows:
   - The required final DTT concentration is 40 mM in BT Tissue Lysis Buffer.
   - To prepare directly before use, add 40 µL 1 M DTT per 1 mL BT Tissue Lysis Buffer and use immediately.
   - Add 250 µL Lysis master mix per sample.
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: Tissue samples contain 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, mix by vortexing or pipetting up and down 20 times. 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 and continue to step 7 using the cleared lysate.
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.

   Note: BT Binding Buffer must be diluted with 100% isopropanol prior to use. Please see Page 5 for instructions.
9. Vortex at maximum speed for 10 minutes.
10. 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.

11. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CBT.
12. Remove the plate containing the CleanNA Particles CBT from the magnetic separation device.
13. 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 5 for instructions.
14. Resuspend the CleanNA Particles CBT by pipetting up and down 20 times or vortexing for 15 seconds.

    Note: Complete resuspension of the CleanNA Particles CBT is critical for obtaining good purity.
15. 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.
16. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CBT.
17. Remove the plate containing the CleanNA Particles CBT from the magnetic separation device.
18. Repeat Steps 13-17 once for a second BT Wash Buffer 1 wash step.
19. 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 5 for instructions.
20. Resuspend the CleanNA Particles CBT by pipetting up and down 20 times or vortexing for 15 seconds.

    Note: Complete resuspension of the CleanNA Particles CBT is critical for obtaining good purity.
21. Incubate at room temperature for 1 minute.
22. 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.
23. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CBT.
24. Leave the plate on the magnetic separation device and select either of the following ethanol removal steps
    - **a.** Leave the plate on the magnetic separation device and add 500 µL molecular biology grade water (not provided) to the samples. Leave on the magnet for 20-30 second and the aspirate to remove the water from the CleanNA particles CBT.
      Do not leave molecular biology grade water on CleanNA Particles CBT for more than 60 seconds.
      Continue to step 25.

      OR
    - **b.** Leave the plate on the magnetic separation device and wait for 1 minute. Remove any residual liquid from the wells using a pipet. While leaving the plate on the magnet, dry the CleanNA Particles CBT for 10 minutes at room temperature.
      Continue to step 25.
25. Remove the plate containing the CleanNA Particles CBT from the magnetic separation device.
26. Add 100-200 µL Elution Buffer or molecular biology grade water to elute DNA from the CleanNA Particles CBT.

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

28. Resuspend the CleanNA Particles CBT by pipetting up and down 30 times or vortexing for 5 minutes.
29. 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 clean microplate (not supplied). Store the DNA at -20°C.

Protocol for Buccal Swabs

This protocol has been optimized for the extraction of genomic DNA from Buccal Swabs. The protocol describes the usage of 96-well round bottom plates, but alternatively single 2 mL tubes with a compatible magnet stand can be used.

Before Starting:

- Prepare all Reagents according to Preparing Reagents section on Page 5.
- Set shaking water bath to 55°C.
- Optionally, set a heat block, incubator or water bath to 70˚C.

Protocol:

1. Cut off the buccal brush or swab head and place each swab into a well of a 96-well deep-well plate.
2. Create a mastermix of BT Lysis Buffer, Elution Buffer and Proteinase K Solution, for the number of samples to be extracted according the table below:

Component	Amount per sample	Total volume for 96-well plate
BT Lysis Buffer	290 µL	30.6 ml
Proteinase K Solution	20 µL	2.1 ml*
Elution Buffer	250 µL	26.4 ml*

* Includes 10% excess volume for a 96-well plate

Note: Only prepare the volume of lysis mastermix required for the number of samples to be extracted. The lysis mastermix can be stored for a maximum of 4 hours until usage.

3. Add 560 µL Lysis mastermix to each buccal swab.
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 2-3 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: Buccal swabs contain 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, mix by vortexing or pipetting up and down 20 times. Incubate at room temperature for 2 minutes.

7. Add 350 µL BT Binding Buffer and 20 µL CleanNA Particles CBT to each sample.

Note: BT Binding Buffer must be diluted with 100% isopropanol prior to use. Please see Page 5 for instructions. BT Binding Buffer and CleanNA Particles CBT can be prepared as a mastermix. Mix only what is needed.

8. 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 5 for instructions.

13. Resuspend the CleanNA Particles CBT by pipetting up and down 20 times or vortexing for 30 seconds.

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 5 for instructions.

19. Resuspend the CleanNA Particles CBT by pipetting up and down 20 times or vortexing for 30 seconds.

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

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 and wait for 1 minute. Remove any residual liquid from the wells using a pipet. While leaving the plate on the magnet, dry the CleanNA Particles CBT for 10 minutes at room temperature.
24. Remove the plate containing the CleanNA Particles CBT from the magnetic separation device.
25. Add 100-200 µL Elution Buffer or molecular biology grade water to elute DNA from the CleanNA Particles CBT.

Note: Heat Elution Buffer or molecular biology grade water to 70°C to improve yield.

26. Resuspend the CleanNA Particles CBT by pipetting up and down 30 times or vortexing for 5 minutes.
27. Incubate at room temperature for 5 minutes.
28. 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.
29. Transfer the cleared supernatant containing purified DNA to a 96-well microplate (not provided). Store DNA at -20°C.

Protocol for Saliva

This protocol has been optimized for the extraction of genomic DNA from Saliva. The protocol describes the usage of 96-well round bottom plates, but alternatively single 2 mL tubes with a compatible magnet stand can be used.

Before Starting:

- Prepare all Reagents according to Preparing Reagents section on Page 5.
- Set shaking water bath to 55°C.
- Optionally, set a heat block, incubator or water bath to 70˚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 500 µL stabilized saliva samples (e.g. DNA Genotek Oragene®, Mawi iSWAB™, Biomatrica® DNAgard® Saliva) into a 96-well deep-well plate.
3. Create a mastermix of BT Lysis Buffer and Proteinase K Solution, for the number of samples to be extracted according the table below:

Component	Amount per sample	Total volume for 96-well plate
BT Lysis Buffer	200 µL	21.1 ml*
Proteinase K Solution	20 µL	2.1 ml*

* Includes 10% excess volume for a 96-well plate

4. Add 220 µL Lysis mastermix to each sample. Vortex 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. Incubate at 55˚C in a shaking water bath for 10 minutes.

   Optional: Saliva samples contain 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, mix by vortexing or pipetting up and down 20 times. Incubate at room temperature for 2 minutes.

6. 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 100% isopropanol prior to use. Please see Page 5 for instructions. BT Binding Buffer and CleanNA Particles CBT can be prepared as a mastermix. Mix only what is needed.

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 5 for instructions.

11. Resuspend the CleanNA Particles CBT by pipetting up and down 20 times or vortexing for 30 seconds.

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

17. Resuspend the CleanNA Particles CBT by pipetting up and down 20 times or vortexing for 30 seconds.
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 and select either of the following ethanol removal steps
    - **a.** Leave the plate on the magnetic separation device and add 500 µL molecular biology grade water (not provided) to the samples. Leave on the magnet for 20-30 second and the aspirate to remove the water from the CleanNA particles CBT.
      Do not leave molecular biology grade water on CleanNA Particles CBT for more than 60 seconds.
      Continue to step 22.

      OR

    - b. Leave the plate on the magnetic separation device and wait for 1 minute. Remove any residual liquid from the wells using a pipet. While leaving the plate on the magnet, dry the CleanNA Particles CBT for 10 minutes at room temperature. Continue to step 22.
22. Remove the plate containing the CleanNA Particles CBT from the magnetic separation device.
23. Add 100-200 µL Elution Buffer or molecular biology grade water to elute DNA from the CleanNA Particles CBT.

    Note: Heat Elution Buffer or molecular biology grade water to 70°C to improve yield.

24. Resuspend the CleanNA Particles CBT by pipetting up and down 30 times or vortexing for 5 minutes.
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.

Protocol for Mouse Tail Snips

This protocol has been optimized for the extraction of genomic DNA from Mouse Tail Snips. The protocol describes the usage of 96-well round bottom plates, but alternatively single 2 mL tubes with a compatible magnet stand can be used.

Before Starting:

- Prepare all Reagents according to Preparing Reagents section on Page 5.
- Set shaking water bath to 55°C.
- Optionally, set a heat block, incubator or water bath to 70˚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 -80°C until DNA is extracted.

2. Add 250 µL BT Tissue Lysis Buffer.

Optional: For lysis of tough-to-lyse tissues, such as hair, a master mix of BT Tissue Lysis Buffer and DTT (not provided) is recommended. Create a BT Tissue Lysis Buffer/DTT master mix as follows:

- The required final DTT concentration is 40 mM in BT Tissue Lysis Buffer.
- To prepare directly before use, add 40 µL 1 M DTT per 1 mL BT Tissue Lysis Buffer and use immediately.
- Add 250 µL Lysis master mix per sample.

3. Add 20 µL of Proteinase K Solution. Vortex to mix thoroughly.
4. Incubate at 55°C in a shaking water bath 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, mix by vortexing or pipetting up and down 20 times. 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.

   Note: BT Binding Buffer must be diluted with 100% isopropanol prior to use. Please see Page 5 for instructions. BT Binding Buffer and CleanNA Particles CBT can be prepared as a mastermix. Mix only what is needed.

9. Vortex at maximum speed for 10 minutes.
10. 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.
11. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CBT.
12. Remove the plate containing the CleanNA Particles CBT from the magnetic separation device.
13. 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 5 for instructions.
14. Resuspend the CleanNA Particles CBT by pipetting up and down 20 times or vortexing for 30 seconds.

    Note: Complete resuspension of the CleanNA Particles CBT is critical for obtaining good purity.
15. 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.
16. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CBT.
17. Remove the plate containing the CleanNA Particles CBT from the magnetic separation device.
18. Repeat Steps 13-17 for a second BT Wash Buffer 1 wash step.
19. 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 5 for instructions.
20. Resuspend the CleanNA Particles CBT by pipetting up and down 20 times or vortexing for 30 seconds.

    Note: Complete resuspension of the CleanNA Particles CBT is critical for obtaining good purity.
21. Incubate at room temperature for 1 minute.
22. 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.
23. Aspirate and discard the cleared supernatant. Do not disturb the CleanNA Particles CBT.
24. Leave the plate on the magnetic separation device and select either of the following ethanol removal steps
    - **a.** Leave the plate on the magnetic separation device and add 500 µL molecular biology grade water (not provided) to the samples. Leave on the magnet for 20-30 second and the aspirate to remove the water from the CleanNA particles CBT.
      Do not leave molecular biology grade water on CleanNA Particles CBT for more than 60 seconds.
      Continue to step 25.

      OR

    - b. Leave the plate on the magnetic separation device and wait for 1 minute. Remove any residual liquid from the wells using a pipet. While leaving the plate on the magnet, dry the CleanNA Particles CBT for 10 minutes at room temperature.
      Continue to step 25.

25. Remove the plate containing the CleanNA Particles CBT from the magnetic separation device.
26. Add 100-200 µL Elution Buffer or molecular biology grade water to elute DNA from the CleanNA Particles CBT.

Note: Heat Elution Buffer or molecular biology grade water to 70°C to improve yield

27. Resuspend the CleanNA Particles CBT by pipetting up and down 30 times or vortexing for 5 minutes.
28. Incubate at room temperature for 5 minutes.
29. 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.
30. Transfer the cleared supernatant containing purified DNA to a 96-well microplate (not provided). Store DNA at -20°C.

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. The protocol has been described using 96-well round bottom plates, but alternatively single 2 mL tubes with a compatible magnet stand can also be used.

Before Starting:

- Prepare all Reagents according to Preparing Reagents section on Page 5.
- Set shaking water bath to 55°C.
- Optionally, set a heat block, incubator or water bath to 70˚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 10^6 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. Create a mastermix of BT Lysis Buffer and Proteinase K Solution, for the number of samples to be extracted according the table below:

Component	Amount per sample	Total volume for 96-well plate
BT Lysis Buffer	230 µL	24.3 ml*
Proteinase K Solution	20 µL	2.1 ml*

* Includes 10% excess volume for a 96-well plate

3. Add 250 µL Lysis mastermix to each sample. Vortex for 10 minutes or pipet up and down 20 times. 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: Cultured cells contain 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, mix by vortexing or pipetting up and down 20 times. Incubate at room temperature for 2 minutes.

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 100% isopropanol prior to use. Please see Page 5 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 5 for instructions.

12. Resuspend the CleanNA Particles CBT by pipetting up and down 20 times or vortexing for 30 seconds.

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 5 for instructions.

18. Resuspend the CleanNA Particles CBT by pipetting up and down 20 times or vortexing for 30 seconds.

Note: BT Wash Buffer 2 must be diluted with ethanol prior to use. Please see Page 5 for instructions.

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 and select either of the following ethanol removal steps
    - **a.** Leave the plate on the magnetic separation device and add 500 µL molecular biology grade water (not provided) to the samples. Leave on the magnet for 20-30 second and the aspirate to remove the water from the CleanNA particles CBT.
      Do not leave molecular biology grade water on CleanNA Particles CBT for more than 60 seconds.
      Continue to step 23.

      OR

    - b. Leave the plate on the magnetic separation device and wait for 1 minute. Remove any residual liquid from the wells using a pipet. While leaving the plate on the magnet, dry the CleanNA Particles CBT for 10 minutes at room temperature. Continue to step 23.
23. Remove the plate containing the CleanNA Particles CBT from the magnetic separation device.

24. Add 100-200 µL Elution Buffer or molecular biology grade water to elute DNA from the CleanNA Particles CBT.

Note: Heat Elution Buffer or molecular biology grade water to 70°C to improve yield.

25. Resuspend the CleanNA Particles CBT by pipetting up and down 30 times or vortexing for 5 minutes.
26. Incubate at room temperature for 5 minutes.
27. Place the plate on the magnetic separation device to magnetize the CleanNA Particles CBT.
28. Incubate at room temperature until the CleanNA Particles CBT are completely cleared from solution.
29. 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 Solutions

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 not released from CleanNA Particles CBT during elution.	Increase elution volume and Incubate at 70°C for 15 minutes; pipette up and down 50 to 100 times.
	Incomplete resuspension of CleanNA Particles CBT during washing.	Fully resuspend the CleanNA Particles CBT during washing steps.
	DNA washed off during wash steps.	Make sure to add ethanol to both BT Wash Buffers 1 and 2 (see Page 5 for instructions).
	Frozen blood samples not mixed properly after thawing.	Completely 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.
	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.

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

Document Revision History

Manual Version	Date of revision	Revised Chapter	Explanation of revision
6.00	October 2021	Total revision.	Overall clearer language.
		All protocols.	Included lysis mastermix tables to the protocols.
		Protocols for Buccal swabs, Saliva and cultured cells.	In mastermix table (step 2 of protocol) changed the unit from µL to ml in the "Total volume for 96 well plate" column.
		Protocol for Mouse Tail Snips.	Added the optional usage of DTT for tough to lyse sample materials.
5.00	August 2020	Total revision.	New lay-out.
		User manual information.	General heading before contents added.

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