Clean Blood & Tissue DNA Kit

Instructions for Use

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REF CBT-D0096, CBT-D0384

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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.

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.

Schematic Overview

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.

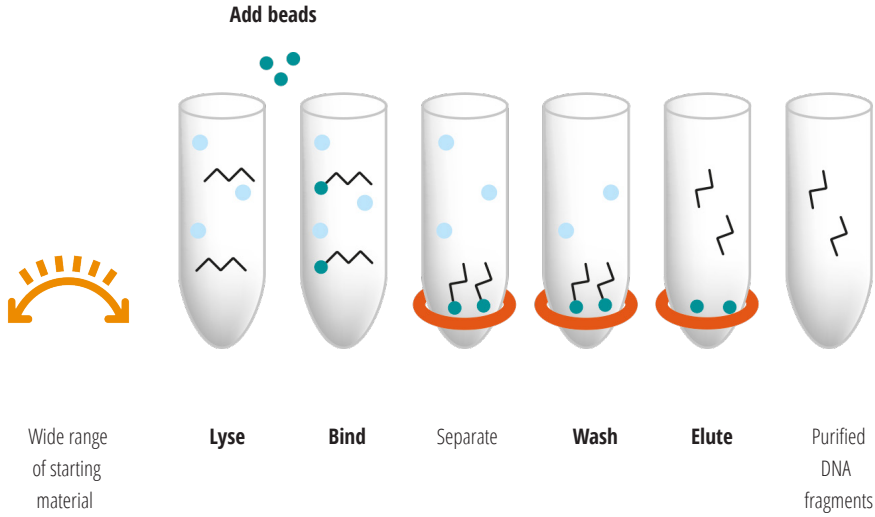

Figure 1: Schematic overview of the Clean Blood & Tissue DNA Kit extraction procedure.

Materials Provided

Kit Contents:

Component	CBT-D0096 (1x96 preps)	CBT-D0384 (4x96 preps)
BT Tissue Lysis Buffer	30 mL	120 mL
BT Lysis Buffer	35 mL	125 mL
BT Binding Buffer	10 mL	40 mL
BT Wash Buffer 1	55 mL	220 mL
Proteinase K Solution (20 mg/mL)	2.2 mL	9 mL
BT Wash Buffer 2	30 mL	120 mL
Elution Buffer	50 mL	200 mL
CleanNA Particles CBT	2.2 mL	9 mL

Reagent Shipping, Storage and Handling

Clean Blood & Tissue DNA Kit is shipped at room temperature (15-25 °C). Do not freeze the components of the Clean Blood & Tissue DNA Kit. After the components have been frozen, the kit is no longer suitable for use. Do not use the Clean Blood & Tissue DNA Kit after the expiration date stated on the outer box label.

Component	Storage Temperature
BT Tissue Lysis Buffer	15-25 °C
BT Lysis Buffer	15-25 °C
BT Binding Buffer	15-25 °C
BT Wash Buffer 1	15-25 °C
Proteinase K Solution (20 mg/mL)	15-25 °C (for storage > 12 months, store at 2-8 °C)
BT Wash Buffer 2	15-25 °C
Elution Buffer	15-25 °C
CleanNA Particles CBT	2-8 °C

⚠ **Note:** Check all buffers for precipitates prior to usage. Any precipitates can be re-dissolved by warming the buffer(s) to 37ºC and shaking gently.

Warnings

Read the instructions carefully before using the kit.

Do not mix several kit LOT numbers.

The LOT number on the CleanNA Particles CBT box packaging is different from the LOT number on the CleanNA Particles CBT bottle. The LOT number on the box matches the LOT number of the whole kit and the one on the bottle is specifically for the particles. Since the CleanNA Particles CBT are stored at a different temperature than the rest of the kit, please make sure that the LOT number on the box packaging of the particles matches the LOT number of the kit before use.

Precautions

When working with chemicals, always follow your facility's procedures and universal precautions by using disposable gloves, safety glasses, a labcoat etc.

For all safety information, please consult the safety data sheet (SDS).

BT Lysis Buffer	
	Harmful if swallowed. Causes skin irritation. Causes serieus eye irritation. Toxic if inhaled. Avoid breathing dust/fume/gas/mist/vapours/spray. **IF IN EYES:** Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. continu rinsing. Call a POISOIN CENTER or doctor/ physician.
BT Binding Buffer	
	May cause fire or explosion; strong oxidizer. May be harmful if swallowed. Wear protective gloves/ protective clothing/ eye protection/ face protection. **IF IN EYES:** Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or doctor/ physician.
Proteinase K Solution	
	May cause an allergic skin reaction. Causes skin irritation. Causes serious eye irritation. May cause allergy or asthma symptoms or breathing difficulties if inhaled. May cause respiratory irritation. Wear protective gloves/protective clothing/eye protection/face protection. Avoid breathing dust/fume/gas/mist/vapours/spray. **IF IN EYES:** Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. If experiencing respiratory symptoms: Call a POISON CENTER or doctor/physician.
BT Wash Buffer 1	
	Harmful if swallowed. Causes skin irritation. Causes serious eye irritation. Toxic if inhaled. Avoid breathing dust/fume/gas/mist/vapours/spray. **IF IN EYES:** Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Call a POISON CENTER or doctor/physician.

Note: For safe disposal, please consult your local waste regulations.

Quality Control

CleanNA produces each lot of Clean Blood & Tissue DNA Kit according to predetermined and validated protocols in the Quality Management System (QMS). Additionally, a quality check after production of each lot is performed to secure consistent product quality. CleanNA's QMS is EN-ISO 13485 certified.

Materials and Equipment to be Supplied by User

General materials and reagents to be supplied by user:

- Absolute ethanol
- Absolute isopropanol (IPA)
- Magnetic separation device for 96-well plates from CleanNA (Cat# CMAG-96-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 Buccal Swabs and Mouse Tail Snips 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 absolute isopropanol (IPA) as follows and store at room temperature.

Kit	Absolute isopropanol to be added
CBT-D0096	40 mL
CBT-D0384	160 mL

BT Wash Buffer 1

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

Kit	Absolute ethanol to be added
CBT-D0096	70 mL
CBT-D0384	280 mL

BT Wash Buffer 2

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

Kit	Absolute 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.

Blood Protocol - up to 250 µL

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 10.
• 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 blood sample 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 must be diluted with isopropanol prior to use. Please see Page 10 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 10 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 10 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, the 10 minutes incubation can be performed at 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.

Tissue Samples Protocol - 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 10.
- 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 absolute isopropanol prior to use. Please see Page 10 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 10 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 10 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.

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

Buccal Swabs Protocol

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 10.
- 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 absolute isopropanol prior to use. Please see Page 10 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 10 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 10 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.

Saliva Protocol

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 10.
- 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 absolute isopropanol prior to use. Please see Page 10 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 10 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:** BT Wash Buffer 2 must be diluted with ethanol prior to use. Please see Page 10 for instructions.

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 10.
- 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 absolute isopropanol prior to use. Please see Page 10 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 10 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 13-17 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 10 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 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.

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.

Cultured Cells Protocol - 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 10.
- 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 cellsin 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.

⚠ **Note:** BT Binding Buffer must be diluted with absolute isopropanol prior to use. Please see Page 10 for instructions. BT Binding Buffer and CleanNA Particles CBT can be prepared as a mastermix. Mix only what is needed.

7. Vortex at maximum speed for 10 minutes.

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 10 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 10 for instructions.

18. 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.

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 Suggestions

Problem	Cause	Suggestion
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 10 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.

Symbols

	Reference number
	Manufacturer
	Caution
	Temperature limit
	Expiration date
	Lot number

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 (384 preps)	CBT-D0384

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

Document Revision History

Manual Version	Date of revision	Revised Chapter	Explanation of revision
7	17/OCT/2023	Total revision.	New lay-out. Added chapters schematic overview, warnings, pre-cautions, quality control, symbols.
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 Tails 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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