MAGBIO

"WE MAKE NGS BETTER"

HighPrep™ Blood & Tissue DNA Kit

Manual Revision 2
Catalog Nos. HPBTS-D5, HPBTS-D96, HPBTS-D96X4

- Genomic DNA isolation from 20-250 µl of blood, lysate of tissues, mouse tails, cultured cells, or buccal swabs
- Magnetic beads based chemistry

PROTOCOL

Contents

Product Description and Process..... 1
Kit Contents, Storage, Stability..... 1
Preparation of Reagents 2
Blood or Saliva DNA 20 -100 µL - 96 well protocol 3
Blood or Saliva DNA 100 -250 µL - 96 well protocol 5
Total DNA from Tissue - 96 well protocol 8
Total DNA from Mouse Tail - 96 well protocol 10
Total DNA from Cultured Cells - 96 well protocol 12
Total DNA from Buccal Swabs - 96 well protocol 14

For Research Use Only. Not for use in diagnostic procedures.

Information in this document is subject to change without notice.

Product Description

The HighPrep™ Blood & Tissue DNA Kit is a high quality genomic DNA purification kit for a variety of sample sources including: fresh or frozen whole blood, buffy coat containing anticoagulants such as Citrate, EDTA and Heparin, DNA from saliva samples, fresh or frozen animal tissues, and cells. Up to 96 samples of tissues can be processed in less than an hour. The kit utilizes our proprietary magnetic beads chemistry and requires no phenol or chloroform extraction or alchohol precipitation, and is suited for high throughput automation. The purified high quality genomic DNA is suitable for direct use in most downstream applications such as amplification and enzymatic reactions.

Process

The HighPrep™ Blood & Tissue DNA Kit uses a simple 3 step procedure: Lyse+Bind-Wash-Elute. Samples are lysed and DNA binds to the MAG-S1 magnetic beads in one step. Utilizing a magnetic separation device, the bound genomic DNA is separated from the solution and washed. The final step is elution of high quality genomic DNA from the magnetic beads.

Kit Contents and Storage

HighPrep™ Blood & Tissue DNA Kit Catalog No.	**HPBTS-D5**	**HPBTS-D96**	**HPBTS-D96X4**	**STORAGE**
Number of Preps	**5**	**96**	**384**	
AS Buffer	2 ml	33 ml	125 ml	15-25°C
TS Buffer	2.5 ml	40 ml	160 ml	15-25°C
HSW Buffer [1]	1.6 ml	22 ml	88 ml	15-25°C
MB Elution Buffer	1 ml	40 ml	120 ml	15-25°C
Pro K Solution [2]	125 µl	2.5 ml	10 ml	2-8°C
MAG-S1 Particles	55 µl	1.1 ml	4.4 ml	2-8°C

[1] Ethanol must be added prior to use. See Preparation of Reagents

Stability

All components are stable for 14 months when stored accordingly.
[2]Pro K Solution comes in a ready to use solution and is stable for 1 year when stored at 15-25°C. For storage longer than 1 year, storage at 2-8°C is recommended.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). MSDS can be downloaded from the "Product Resource" tab when viewing the product kit.

Preparation of Reagents

Prepare the following components for each kit before use:

Catalog No.	Component	Add 100% Ethanol	Storage
HPBTS-D5	HSW Buffer	1.46 ml	Room Temp 15-25°C
Components are stable for 14 months when stored closed at room temperature			

Catalog No.	Component	Add 100% Ethanol	Storage
HPBTS-D96	HSW Buffer	28 ml	Room Temp 15-25°C
Components are stable for 14 months when stored closed at room temperature			

Catalog No.	Component	Add 100% Ethanol	Storage
HPBTS-D96X4	HSW Buffer	112 ml	Room Temp 15-25°C
Components are stable for 14 months when stored closed at room temperature			

Amounts of starting material

Use the amounts of starting material indicated in Table 1.

Blood Sample	**Amount**
Blood	20 - 250 µL
Buffy coat	20 - 250 µL
Saliva	20 - 250 µL
Tissue Sample	**Amount**
Most tissue samples	10 mg
Spleen	5 to 6 mg

Protocol: Total DNA from Whole Blood 20 -100 µL (96 well format)

The following protocol can be applied for saliva samples.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs) from each product supplier.

- ☐ 500 µL 96-well round bottom plate or desired elution plate
- ☐ 1.2 mL deep-well plate
- ☐ 96 magnetic separation device for 1.2 mL deep well plate
- ☐ Sealing film for storage
- ☐ 70% Ethanol
- ☐ 100% Ethanol
- ☐ Optional: Phosphate-buffered saline (PBS) or nuclease-free water may be required
- ☐ Optional: RNase A (10 mg/mL)

Things to do before starting

- ☐ Equilibrate samples to room temperature.
- ☐ Ensure HSW Buffer is prepared according to the instructions on page 2 and is at room temperature.
- ☐ AS Buffer and TS Buffer may show precipitates during storage. If precipitates are present, heat bottle to 37°C to dissolve the precipitates before use.

Protocol

1. Add 20 -100 µL of the sample to a 1.2 mL deep-well plate. Bring the sample volume up to 200 µL with PBS or included **MB Elution Buffer.**

2. Add 20 µL of **Pro K Solution** to the sample and pipette mix 20 times or vortex for 15 sec.

3. Optional: Add 5 µL of RNase A to the sample and pipette mix 20 times or vortex for 15 sec.

4. Add 200 µL of **AS Buffer** to the sample and pipette mix 20 times.

5. Incubate the sample plate at 65°C for 30 min. Mix by inverting the plate once every 10 min during incubation.

6. Bring the sample plate to room temperature.

7. Add 300 µL of **100% Ethanol** and 10 µL of **MAG-S1 Particles** to the sample, and pipette mix 20 times.

   ⚠ *Shake thoroughly the MAG-S1 Particles to fully resuspend before use.*

8. Incubate the sample plate at room temperature for 5 min.

9. Transfer 360 µL of the sample to a new 96 well processing microplate with a capacity of at least 500 µL.

10. Place the sample processing plate containing the samples on the magnetic separation device for 5 min to magnetize the **MAG-S1 Particles.**

11. With the plate on the magnetic separation device, remove and discard the supernatant by pipetting.

    ⚠ *Do not disturb the attracted beads while aspirating the supernatant.*

12. Remove the sample processing plate from the magnetic separation device. Repeat steps 9 to 11 until all the samples from the 1.2mL sample plate are transferred to the new sample processing plate.

13. With the plate off the magnetic separation device, add 400 µL of **HSW Buffer** to the sample and mix by pipetting 25 times or vortex for 1 min to resuspend the **MAG-S1 Particles.**

    ⚠ *Complete resuspension of the MAG-S1 Particles is crucial for obtaining high purity DNA.*

14. Place the sample plate back on the magnetic separation device and wait for 5 min or until the magnetic beads clear from solution.

15. With the plate on the magnetic separation device, remove and discard the supernatant by pipetting.

    ⚠ *Do not disturb the attracted beads while aspirating the supernatant.*

16. Remove the plate off the magnetic separation device, add 400 µL of **70% Ethanol** to the sample, and mix by pipetting 25 times or vortex for 1 min to resuspend the **MAG-S1 Particles.**

17. Incubate at room temperature for 1 min.

18. Place the sample processing plate containing the sample on the magnetic separation device for 5 min to magnetize the **MAG-S1 Particles**.

19. With the plate on the magnetic separation device, remove and discard the supernatant by pipetting.

20. Repeat steps 16 to 19 for a second Ethanol wash.

21. Dry the beads by incubating for 10 min at room temperature with the plate still on the magnetic separation device.

    ⚠ *Do not overdry the beads.*

22. Remove the plate from the magnetic separation device. Add 100 - 200 µL of **MB Elution Buffer** or nuclease-free water to the sample and mix 50 times or vortex for 2 min to completely resuspend the **MAG-S1 Particles.**

    ⚠ *Complete resuspension of the MAG-S1 Particles is crucial for obtaining high purity DNA.*

23. Incubate at room temperature for 10 min.

24. Place the sample plate back on the magnetic separation device and wait for 5 min or until the magnetic beads clear from the solution.

25. Transfer the eluate (cleared supernatant containing the DNA) to a microplate for storage. Store DNA at -20°C.

Protocol: Total DNA from Whole Blood 100 - 250 µL (96 well format)

The following protocol can be applied for saliva samples.

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs) from each product supplier.

- ☐ 500 µL 96-well round bottom plate or desired elution plate
- ☐ 1.2 mL deep-well plate
- ☐ 96 magnetic separation device for 1.2 mL deep well plate
- ☐ Sealing film for storage
- ☐ 70% Ethanol
- ☐ 100% Ethanol
- ☐ Optional: Phosphate-buffered saline (PBS) or nuclease-free water may be required
- ☐ Optional: RNase A (10 mg/mL)

Things to do before starting

- ☐ Equilibrate samples to room temperature.
- ☐ Ensure HSW Buffer is prepared according to the instructions on page 2 and is at room temperature.
- ☐ AS Buffer and TS Buffer may show precipitates during storage. If precipitates are present, heat the bottle to 37°C to dissolve the precipitates before use.

Protocol

1. Add 100 - 250 µL of the sample to a 1.2 mL deep-well plate. Bring sample volume up to 300 µL with PBS or with included **MB Elution Buffer.**

2. Add 20 µL of **Pro K Solution** to the sample and pipette mix 20 times or vortex for 15 sec.

3. Optional: Add 5 µL of RNase A to the sample and pipette mix 20 times or vortex for 15 sec.

4. Add 300 µL of **AS Buffer** to the sample and pipette mix 20 times.

5. Incubate the sample plate at 65°C for 30 min. Mix by inverting the plate once every 10 min during incubation.

6. Bring the sample plate to room temperature.

7. Add 430 µL of **100% Ethanol** and 10 µL of **MAG-S1 Particles** to the sample, and pipette mix 20 times.

   ⚠*Shake thoroughly the MAG-S1 Particles to fully resuspend before use.*

8. Incubate the sample plate at room temperature for 5 min.

9. Transfer 360 µL of the sample to a new 96 well processing microplate with a capacity of at least 500 µL.

10. Place the sample processing plate containing the sample on the magnetic separation device for 5 min to magnetize the **MAG-S1 Particles.**

11. With the plate on the magnetic separation device, remove and discard the supernatant by pipetting.

    ⚠ *Do not disturb the attracted beads while aspirating the supernatant*

12. Remove the sample processing plate from the magnetic separation device. Repeat steps 9 to 11 until all the sample from the 1.2mL sample plate is transferred to the new sample processing plate.

13. With the plate off the magnetic separation device, add 400 µL of **HSW Buffer** to the sample and mix by pipetting 25 times or vortex for 1 min to resuspend the **MAG-S1 Particles.**

    ⚠ *Complete resuspension of the MAG-S1 Particles is crucial for obtaining high purity DNA.*

14. Place the sample plate back on the magnetic separation device and wait for 5 min or until the magnetic beads clear from solution.

15. With the plate on the magnetic separation device, remove and discard the supernatant by pipetting.

    ⚠ *Do not disturb the attracted beads while aspirating the supernatant.*

16. Remove the plate off the magnetic separation device, add 400 µL of **70% Ethanol** to the sample and mix by pipetting 20 times or vortex for 1 min to resuspend the **MAG-S1 Particles.**

17. Incubate at room temperature for 1 min.

18. Place the sample processing plate containing the sample on the magnetic separation device for 5 min to magnetize the **MAG-S1 Particles.**

19. With the plate on the magnetic separation device, remove and discard the supernatant by pipetting.

20. Repeat steps 16 to 18 for a second Ethanol wash.

21. Dry the beads by incubating for 10 min at room temperature with the plate still on the magnetic separation device.

22. Remove the plate from the magnetic separation device. Add 100 - 200 µL of **MB Elution Buffer** or nuclease-free water to the sample and mix 50 times or vortex for 2 min to completely resuspend the **MAG-S1 Particles.**

    ⚠ *Complete resuspension of the MAG-S1 Particles is crucial for obtaining high purity DNA.*

23. Incubate at room temperature for 10 min.

24. Place the sample plate back on the magnetic separation device and wait for 5 min or until the magnetic beads clear from solution.

25. Transfer the eluate (cleared supernatant containing the DNA) to a microplate for storage. Store DNA at -20°C.

Troubleshooting guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact technical support via:
Phone: 1-855-262-4246 (in US), outside US, 1-301-302-0144
Email: support@magbiogenomics.com

Symptoms	Possible Causes	Comments
Low DNA yield	Frozen samples not mixed properly after thawing	Thaw the frozen samples at room temperature and gently mix the samples by inverting.
	Blood is too old	Best yields are obtained from fresh blood.
	Low levels of leukocytes	Low white blood cells count will give reduced yield.
	Incomplete resuspension of MAG-S1 Particles	Resuspend MAG-S1 Particles by vortexing vigorously before use.
	Loss of MAG-S1 Particles during operation	Avoid disturbing the MAG-S1 Particles during aspiration of supernatant.
	DNA remains bound to MAG-S1 Particles	Increase elution volume and incubate for 15 minutes. Pipet up and down 50 to 100 times.
	Ethanol is not added into HSW Buffer	Add absolute 100% Ethanol to HSW Buffer (see page 2 for instructions).
MAG-S1 Particles do not completely clear from solution	Too short of magnetizing time	Increase collection time on the magnet.
Eluted DNA contains gelatinous material	Blood is too old	Remove the gelatinous material by centrifugation. Recommend using fresh blood.
		Use 8 mM NaOH as elution buffer.
Problems in downstream applications	Ethanol carry-over	Dry the MAG-S1 Particles completely before elution.

Protocol: Total DNA from Animal Tissues - 96 well format

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs) from each product supplier.

- ☐ 96 well round-bottom deep well plates with a capacity of 1 mL per well (ABgene AB-1127)
- ☐ Magnetic separation device compatible with 96-well plate
- ☐ Centrifuge with swing bucket rotor capable of 4,000 x g
- ☐ Shaking water bath
- ☐ Vortex
- ☐ 70% Ethanol
- ☐ 100% Ethanol
- ☐ Optional RNase A (10 mg/mL)

Things to do before starting

- ☐ Ensure HSW Buffer is prepared according to the instructions on page 2 and is at room temperature.
- ☐ Warm up MB Elution Buffer to 70°C.
- ☐ Set shaking water bath to 55°C.
- ☐ AS Buffer and TS Buffer may show precipitates during storage. If precipitates are present, heat the bottle to 37°C to dissolve the precipitates before use.

Protocol

1. Place up to 10 mg of tissue into a well of a 96 deep-well plate. Add 250 µl of **TS Buffer**.
   Note: Cutting/mincing the tissue into smaller pieces can speed up the lysis process.
   Optional. To improve lysis and reduce incubation time, pulverize sample to fine powder in liquid nitrogen.

   For spleen tissue, use 5-6 mg. This will reduce the thickness of the gDNA extracted solution and allow a more efficient wash and ultimately a better quality extracted DNA.

2. Add 20 µl of **Pro K Solution** to each sample well. Seal the plate and vortex to mix well and incubate at 55°C in a shaking water bath overnight. Overnight lysis is recommended for optimal yield.
   Alternatively, lysis can be performed in 2-4 hours depending on the amount and tissue type.
   If a shaking water bath is not available, vortex the plate every 20-30 min.

3. Quickly spin the plate for 20 seconds to collect liquid.
   For tissues samples containing material that cannot be digested during the lysis step, centrifuge the plate at maximum speed for 5 min to pellet the undigested materials. Transfer the clear lysate on top to a new processing plate.

4. Optional: Add 5 µl of RNase A to each sample well. Pipette mix 20 times or vortex for 15 sec.

5. Add 200 µl of **AS Buffer** to the sample, pipette mix 20 times or vortex for 15 seconds, and incubate at 70°C for 10 min.

6. Bring the sample plate to room temperature and add 290 µl of 100 % Ethanol and 10 µl MAG-S1 particles to the sample, and pipette mix 25 times. Incubate at room temperature for 5 min.

   ⚠ *Shake thoroughly the MAG-S1 Particles to fully resuspend before use.*

7. Place the sample plate on the magnetic separation device to magnetize the **MAG-S1 Particles** and wait for 3 min or until the beads clear from the solution.

8. With the plate on the magnetic separation device, remove and discard the supernatant by pipetting.

   ⚠ *Do not disturb the attracted beads while aspirating the supernatant.*

9. Remove the plate from the magnetic separation device. Add 400 µl of **HSW Buffer** to the sample and pipette mix 25 times or vortex for 30 sec to resuspend the **MAG-S1 Particles.**

   ⚠ *Complete resuspension of the Mag-S1 Particles is crucial for obtaining high purity DNA.*

10. Place the sample plate back on the magnetic separation device and wait for 5 min or until the magnetic beads clear from solution.

11. With the plate on the magnetic separation device, remove and discard the supernatant by pipetting.

    ⚠ *Do not disturb the attracted beads while aspirating the supernatant.*

12. Remove the sample plate from the magnetic device. Add 400 µl of **70% Ethanol** to the sample and pipette mix 25 times or vortex for 1 min to resuspend the **MAG-S1 Particles.** Incubate at room temperature for 3 min.

13. Place the sample plate back on the magnetic separation device and wait for 3 min or until the magnetic beads clear from solution.

14. With the plate on the magnetic separation device, remove and discard the supernatant by pipetting.

    ⚠ *Do not disturb the attracted beads while aspirating the supernatant.*

15. Repeat steps 12-14 for a second 70% Ethanol wash.

16. Dry the beads by incubating for 10 min at room temperature with the plate still on the magnetic separation device.

    ⚠ *It is critical to completely remove all liquid from each well.*

17. Remove the plate from the magnetic separation device. Add 50-200 µl of MB Elution Buffer or nuclease-free water to the sample and pipette mix 50 times or vortex for 2 min to completely resuspend the MAG-S1 particles.

    ⚠ *Complete resuspension of the MAG-S1 Particles is crucial for obtaining high purity DNA.*

18. Incubate at room temperature for 10 min.

    ⚠ *Incubation at 70°C can increase the yield.*

19. Place the sample plate back on the magnetic separation device and wait for 5 min or until the magnetic beads clear from solution.

20. Transfer the eluate (cleared supernatant containing the DNA) to a microplate for storage. Store DNA at -20°C.

Protocol: Total DNA from Mouse Tail - 96 well format

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs) from each product supplier.

- ☐ 96 well round-bottom deep well plates with a capacity of 1 mL per well (ABgene AB-1127)
- ☐ Magnetic separation device compatible with 96-well plate
- ☐ Centrifuge with swing bucket rotor capable of 4,000 x g
- ☐ Shaking water bath
- ☐ Vortex
- ☐ 70% Ethanol
- ☐ 100% Ethanol
- ☐ Optional: RNase A (10 mg/mL)

Things to do before starting

- ☐ Ensure HSW Buffer is prepared according to the instructions on page 2 and is at room temperature.
- ☐ Warm up MB Elution Buffer to 70°C.
- ☐ Set shaking water bath to 55°C.
- ☐ AS Buffer and TS Buffer may show precipitates during storage. If precipitates are present, heat the bottle to 37°C to dissolve the precipitates before use.

1. Take a 2-5 mm piece of mouse tail and mince into several pieces. Add 250 µl of **TS Buffer.**
   Note: Cutting/mincing the tissue into smaller pieces can speed up the lysis process.
   Optional. To improve lysis and reduce incubation time, pulverize sample to fine powder in liquid nitrogen.

2. Add 20 µl of **Pro K Solution**. Vortex to mix well and incubate at 55°C overnight in a shaking water bath. If a shaking water bath is not available, vortex the plate every 20-30 min.
   Lysis time depends on the length of the tail snip and age of the mice. Biopsies should be from 2-4 weeks old mice. For older mice, overnight incubation may improve yields.

3. Centrifuge the plate at maximum speed for 5 min to pellet the undigested materials. Transfer the clear lysate on top to a new processing plate with a capacity of 500 µl per well.

4. Optional: RNA in the mouse tail will be copurified. If the RNA will interfere with your downsteam application, remove the RNA by adding 5 µl RNase A. Pipette mix for 20 times or vortex for 15 sec.

5. Add 200 µl of **AS Buffer** to the sample, pipette mix 20 times or vortex for 15 sec, and incubate at 70°C for 10 min.

6. Bring the sample plate to room temperature and add 290 µl of **100% Ethanol** and 10 µl of **MAG-S1 Particles** to the sample, and pipette mix 25 times. Incubate at room temperature for 5 min.

   ⚠ *Shake thoroughly the MAG-S1 Particles to fully resuspend before use.*

7. Place the sample plate on the magnetic separation device to magnetize the **MAG-S1 Particles** and wait for 3 min or until the beads clear from the solution.

8. With the plate on the magnetic separation device, remove and discard the supernatant by pipetting.

   ⚠ *Do not disturb the attracted beads while aspirating the supernatant.*

9. Remove the plate from the magnetic separation device. Add 400 µl of **HSW Buffer** to the sample and pipette mix 25 times or vortex for 30 sec to resuspend the **MAG-S1 Particles**.

   ⚠ *Complete resuspension of the MAG-S1 Particles is crucial for obtaining high purity DNA.*

10. Place the sample plate back on the magnetic separation device and wait for 5 min or until the magnetic beads clear from solution.

11. With the plate on the magnetic separation device, remove and discard the supernatant by pipetting.

    ⚠ *Do not disturb the attracted beads while aspirating the supernatant.*

12. Remove the sample plate from the magnetic device. Add 400 µl of **70% Ethanol** to the sample and pipette mix 20-25 times or vortex for 1 min to resuspend the **MAG-S1 Particles**. Incubate at room temperature for 3 min.

13. Place the sample plate back on the magnetic separation device and wait for 3 min or until the magnetic beads clear from solution.

14. With the plate on the magnetic separation device, remove and discard the supernatant by pipetting.

    ⚠ *Do not disturb the attracted beads while aspirating the supernatant.*

15. Repeat steps 12-14 for a second 70% ethanol wash.

16. Dry the beads by incubating for 10 min at room temperature with the plate still on the magnetic separation device.

17. Remove the plate from the magnetic separation device. Add 50 - 200 µl of **MB Elution Buffer** or nuclease-free water to the sample and pipette mix 50 times or vortex for 2 min to completely resuspend the **MAG-S1 Particles.**

    ⚠ *Complete resuspension of the MAG-S1 Particles is crucial for obtaining high purity DNA.*

18. Incubate at room temperature for 10 min.

    ⚠ *Incubation at 70°C can increase the yield.*

19. Place the sample plate back on the magnetic separation device and wait for 5 min or until the magnetic beads clear from the solution.

20. Transfer the eluate (cleared supernatant containing the DNA) to a microplate for storage. Store DNA at -20°C

Protocol: Total DNA From Cultured Cells - 96 well Format

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs) from each product supplier.

- ☐ 96 well round-bottom deep well plates with a capacity of 1 mL per well (ABgene AB-1127)
- ☐ Magnetic separation device compatible with 96-well plate
- ☐ Centrifuge with swing bucket rotor capable of 4,000 x g
- ☐ Shaking water bath
- ☐ Vortex
- ☐ Chilled PBS (4°C)
- ☐ 70% Ethanol
- ☐ 100% Ethanol

Things to do before starting

- ☐ Ensure HSW Buffer is prepared according to the instructions on page 2 and is at room temperature.
- ☐ Warm up MB Elution Buffer to 70°C.
- ☐ Set shaking water bath to 55°C.
- ☐ AS Buffer and TS Buffer may show precipitates during storage. If precipitates are present, heat the bottle to 37°C to dissolve the precipitates before use.

Protocol

1. Prepare cultured cells suspension according to your starting sample method:

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 of 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 of 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 of **Pro K Solution**. Vortex or pipette mix thoroughly and incubate at 55°C in a water bath for 10 min.

3. Transfer samples to a new 96-well plate.

4. Add 200 µl of **AS Buffer** to the sample, pipette mix 20 times or vortex for 15 sec, and incubate at 70°C for 10 min.

5. Bring the sample plate to room temperature, add 290 µl of **100% Ethanol** and 10 µl of **MAG-S1 Particles** to the sample, and pipette mix 25 times. Incubate at room temperature for 5 min.

⚠ *Shake thoroughly the MAG-S1 Particles to fully resuspend before use.*

6. Place the sample plate on the magnetic separation device to magnetize the **MAG-S1 Particles** and wait for 3 min or until the beads clear from the solution.

7. With the plate on the magnetic separation device, remove and discard the supernatant by pipetting.

   ⚠ *Do not disturb the attracted beads while aspirating the supernatant.*

8. Remove the plate from the magnetic separation device. Add 400 µl of **HSW Buffer** to the sample and pipette mix 25 times or vortex for 30 sec to resuspend the **MAG-S1 Particles.**

   ⚠ *Complete resuspension of the MAG-S1 Particles is crucial for obtaining high purity DNA.*

9. Place the sample plate back on the magnetic separation device and wait for 5 min or until the magnetic beads clear from solution.

10. With the plate on the magnetic separation device, remove and discard the supernatant by pipetting.

    ⚠ *Do not disturb the attracted beads while aspirating the supernatant.*

11. Remove the sample plate from the magnetic device. Add 400 µl of **70% Ethanol** to the sample and pipette mix 25 times or vortex for 1 min to resuspend the **MAG-S1 Particles**. Incubate at room temperature for 3 min.

12. Place the sample plate back on the magnetic separation device and wait for 3 min or until the magnetic beads clear from solution.

13. With the plate on the magnetic separation device, remove and discard the supernatant by pipetting.

    ⚠ *Do not disturb the attracted beads while aspirating the supernatant.*

14. Repeat steps 11-13 for a second 70% ethanol wash.

15. Dry the beads by incubating for 10 min at room temperature with the plate still on the magnetic separation device.

    ⚠ *It is critical to completely remove all liquid from each well.*

16. Remove the plate from the magnetic separation device. Add 50 - 200 µl of **MB Elution Buffer** or nuclease-free water to the sample and pipette mix 50 times or vortex for 2 min to completely resuspend the **MAG-S1 Particles**.

    ⚠ *Complete resuspension of the MAG-S1 Particles is crucial for obtaining high purity DNA.*

17. Incubate at room temperature for 10 min.

    ⚠ *Incubation at 70°C can increase the yield.*

18. Place the sample plate back on the magnetic separation device and wait for 5 min or until the magnetic beads clear from solution.

19. Transfer the eluate (cleared supernatant containing the DNA) to a microplate for storage. Store DNA at -20°C

Protocol: Total DNA From Buccal Swabs - 96 well Format

Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs) from each product supplier.

- ☐ 96 well round-bottom deep well plates with a capacity of 1 mL per well (ABgene AB-1127)
- ☐ Magnetic separation device compatible with a 96-well plate
- ☐ Centrifuge with swing bucket rotor capable of 4,000 x g
- ☐ Shaking water bath
- ☐ Vortex
- ☐ 70% Ethanol
- ☐ 100% Ethanol

Things to do before starting

- ☐ Ensure HSW Buffer is prepared according to the instructions on page 2 and is at room temperature.
- ☐ Warm up MB Elution Buffer to 70°C.
- ☐ Set shaking water bath to 55°C.
- ☐ AS Buffer and TS Buffer may show precipitates during storage. If precipitates are present, heat the bottle to 37°C to dissolve the precipitates before use.

Protocol

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

2. Add 400 µl of **TS Buffer** to each sample well.

3. Add 25 µl of **Pro K Solution**. Vortex or pipette mix thoroughly and incubate at 55°C in a water bath for 45 min. Mix by inverting the 96 well deep plate once during incubation.

4. Centrifuge the plate at 3,000 x g for 10 min.

5. Transfer 200 µl of lysate to a new 96 well deep plate.

   ⚠ *Do not transfer the swabs or other debris.*

6. Add 200 µl of AS Buffer to the sample, pipette mix 20 times or vortex for 15 sec, and incubate at 70°C for 10 min.

7. Bring the sample plate to room temperature, add 290 µl of **100% Ethanol** and 10 µl of **MAG-S1 Particles** to the sample, and pipette mix 25 times. Incubate at room temperature for 5 min.

   ⚠ *Shake thoroughly the MAG-S1 Particles to fully resuspend before use.*

8. Place the sample plate on the magnetic separation device to magnetize the **MAG-S1 Particles** and wait for 3 min or until the beads clear from the solution.

9. With the plate on the magnetic separation device, remove and discard the supernatant by pipetting.

   ⚠ Do not disturb the attracted beads while aspirating the supernatant.

10. Remove the plate from the magnetic separation device. Add 400 µl of **HSW Buffer** to the sample and pipette mix 25 times or vortex for 30 sec to resuspend the **MAG-S1 Particles**.

    ⚠ *Complete resuspension of the MAG-S1 Particles is crucial for obtaining high purity DNA*.

11. Place the sample plate back on the magnetic separation device and wait for 5 min or until the magnetic beads clear from solution.

12. With the plate on the magnetic separation device, remove and discard the supernatant by pipetting.

    ⚠ *Do not disturb the attracted beads while aspirating the supernatant.*

13. Remove the sample plate from the magnetic device. Add 400 µl of **70% Ethanol** to the sample and pipette mix 25 times or vortex for 1 min to resuspend the **MAG-S1 Particles.** Incubate at room temperature for 3 min.

14. Place the sample plate back on the magnetic separation device and wait for 3 min or until the magnetic beads clear from solution.

15. With the plate on the magnetic separation device, remove and discard the supernatant by pipetting.

    ⚠ *Do not disturb the attracted beads while aspirating the supernatant.*

16. Repeat steps 13-15 for a second 70% Ethanol wash.

17. Dry the beads by incubating for 10 min at room temperature with the plate still on the magnetic separation device.

18. Remove the plate from the magnetic separation device. Add 50 - 200 µl of MB Elution Buffer or nuclease-free water to the sample and pipette mix 50 times or vortex for 2 min to completely resuspend the **MAG-S1 Particles.**

    ⚠ *Complete resuspension of the MAG-S1 Particles is crucial for obtaining high purity DNA*.

19. Incubate at room temperature for 10 min.

    ⚠ *Incubation at 70°C can increase the yield.*

20. Place the sample plate back on the magnetic separation device and wait for 5 min or until the magnetic beads clear from solution.

21. Transfer the eluate (cleared supernatant containing the DNA) to a microplate for storage. Store DNA at -20°C.

Troubleshooting guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact technical support via:
Phone: 1-855-262-4246 (in US), outside US, 1-301-302-0144
Email: support@magbiogenomics.com

Symptoms	Possible Causes	Comments
Low DNA Yield	Incomplete resuspension of MAG-S1 Particles	Resuspend the MAG-S1 Particles by vortexing vigorously before use.
	Loss of MAG-S1 Particles during operation	Avoid disturbing the MAG-S1 Particles during aspiration of supernatant.
	DNA remains bound to the MAG-S1 Particles	Increase elution volume and incubate for 15 minutes. Pipette mix 50 to 100 times.
	Ethanol is not added into HSW Buffer	Add absolute 100% Ethanol to HSW Buffer (see page 2 for instructions).
MAG-S1 particles do not completely clear from solution	Too short of magnetizing time	Increase collection time on the magnet.
Problems in downstream applications	Insufficient DNA in starting material	Use more starting material.
	Ethanol carry-over	Dry the MAG-S1 Particles completely before elution.

Ordering Information

Product Description	Catalog No.	Preps
HighPrep™ Blood & Tissue DNA Kit	HPBTS-D96	96
HighPrep™ Blood & Tissue DNA Kit	HPBTS-D96X4	384

Post PCR and Next Gen library prep clean-up system

Product Description	Catalog No.
HighPrep™ PCR (5 mL)	AC-60005
HighPrep™ PCR (50 mL)	AC-60050
HighPrep™ PCR (520 mL)	AC-60250
HighPrep™ PCR (500 mL)	AC-60500

Magnetic Separation Devices

Catalog No.	Description
MYMAG-96	Handheld Magnetic Separation Device (96 well microplate format)
MYMAG-96X	Magnetic Separation Device (96 well ring format)
MBMS-12	MagStrip magnetic stand (1.5 mL x 12)
MBMS-31550	15ml and 50ml magnetic stand combo. (3x15ml and 3x50ml)

Precautions and Disclaimers

This kit is designed for research purposes only. It is not intended for human or diagnostic use. All biological samples are considered potentially infectious. When working with the samples and chemicals always wear a suitable lab coat, disposable gloves, and protective goggles. For more information please consult the appropriate Material Safety Data Sheets (MSDSs).

www.magbiogenomics.com