SHENTEK

Mycoplasma DNA Extraction Kit (2G)

User Guide

Version: A/2
For Research Use Only
Product No.: 1509840
Reagents for 50 Extractions

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(IMPORTANT: Please read this document carefully before experiment.)

1. Product information

■ Product description

MycoSHENTEK® Mycoplasma DNA Extraction Kit (2G) is used for extraction of trace mycoplasma DNA from master cell banks, working cell banks, virus seed banks, and complex matrices such as 10^7 cells culture, 5% human albumin culture solution, or high-concentration plasmids, etc. It works with MycoSHENTEK® Mycoplasma Detection Kit (2G) for an integrated workflow from sample preparation to detection assay. Validated according to USP 63, EP 2.6.7 and JP XVIII for mycoplasma detection with a detection limit of 10 CFU/mL.

For sample volume that is less than 400 μL, test sample DNA can be extracted directly using Mycoplasma DNA Extraction Kit (2G). If it is necessary to increase the sample volume to achieve a higher detection sensitivity, the sample should be concentrated by centrifugation to a final volume of approximately 400 μL before using this extraction kit.

The kit is compatible with manual sample preparation, or with automated extraction using rHCDpurify system.

■ Kit contents and storage

WARNING: Please read the Material Safety Data Sheets (MSDS) and follow the handling instructions. Wear appropriate protective eyewear, mask, clothing and gloves.

Table 1. Kit component and storage

No.	Reagent	Part No.	Quantity	Storage
I	Wash buffer A	NND015	15 mL × 1 bottle	room temperature
	Binding solution	NND017	10 mL × 1 bottle	
	Elution buffer	NND019	5 mL × 1 bottle	
	Dilution buffer	NND022	5 mL × 1 bottle	
	Lysis buffer	NND028	5 mL × 1 bottle	
II	Pretreatment buffer	NND002	1.25 mL × 4 tubes	2-8℃
	Magnetic particles	NND033	750 μL × 2 tubes	
	5M NaCl	NND040	500 μL × 1 tube	
III	Precipitation solution I	NND003	25 μL × 1 tube	-20℃
	Precipitation solution II	NND004	500 μL × 1 tube	
	Proteinase K	NND023	500 μL × 2 tubes	

The kit components can be stored at the appropriate conditions for up to 24 months. Please check the expiration date on the labels.

■ Required materials not included in the kit

- Ultra-pure water
- Anhydrous Ethanol (AR)
- 100% Isopropanol(AR)
- PCR 8-well strip tubes with caps or 96-well plates with seals
- Low retention, RNase/DNase-free, sterile filter tips: 1000μL, 100μL, 10μL
- Low retention, RNase/DNase-free, sterile microcentrifuge tubes: 1.5mL, 2.0mL, 50mL
- Internal Control (IC) (2G) (NNA035) and DNA Dilution Buffer (DDB) (NND001), components of the Mycoplasma DNA Detection Kit (2G) (Product No. 1509841)

■ Related equipment

- Benchtop microcentrifuge
- Magnetic stand or rHCDpurify system
- High-speed refrigerated centrifuge

- Vortex mixer
- Dry bath incubator
- Pipettes, 1000μL, 100μL, 10μL
- Real-time PCR System
- Laminar flow hood or biosafety cabinet
- Microplate and microtube shaker

2. Methods

■ Experiment preparation

Before first use of the kit:

- Add 20 mL of Anhydrous Ethanol to Wash buffer A (NND015).
- Prepare a 70% Anhydrous Ethanol buffer with ultra-pure water in a clean tube, label as Wash buffer B.
- Store Wash buffer A&B at room temperature (RT) properly to prevent evaporation by expiration date.
- Centrifuge the Internal Control (IC) (2G) lyophilized powder (NNA035) at 16,000×g for 2 min and add 600 μL of DNA Dilution Buffer (DDB) (NND001). Vortex to mix well and centrifuge in a microcentrifuge, and repeat three times.

  Note: IC & DDB are the components of the Mycoplasma DNA Detection Kit (2G).

Before each use of the kit:

- Prepare 100% Isopropanol.
- Set the dry bath temperatures to 55°C (or 25°C) and 70°C.

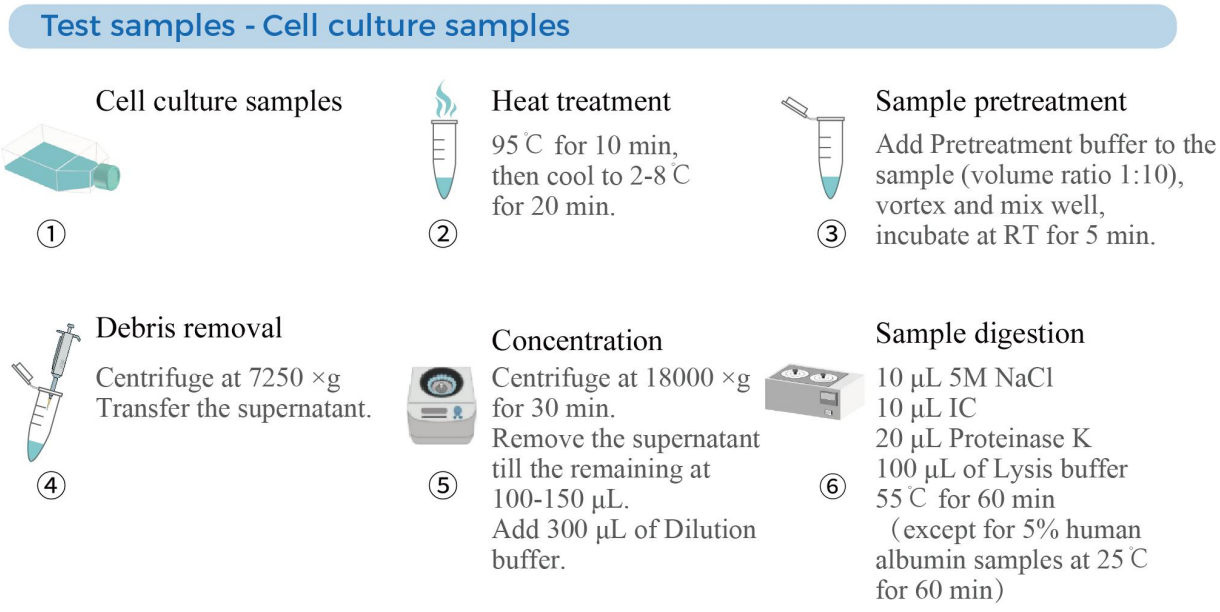
  Note: If the Lysis buffer (NND028) or Binding solution (NND017) is cloudy or contains precipitates, heat at 37°C until it clears.
- Set a refrigerated centrifuge temperature to 2-8°C.
- Place the Magnetic particles (NND030) at room temperature for 10 minutes, vortex for 5 seconds to mix thoroughly before use.

➢ Binding Buffer preparation:

1. Precipitation solution I dilution: According to the volume of Binding Buffer needed for the experiment, prepare the volume of Precipitation solution I (NND003) and dilute it with DDB at a volume ratio of 1:99.
2. Binding Buffer preparation (for one sample): Add 200 µL of Binding solution (NND017), 5 µL of diluted Precipitation solution I (please see Binding Buffer preparation - Step 1) and 9 µL of Precipitation solution II (NND004), and mix well.

Note: Prepare the total volume of binding buffer appropriately more than necessary for experiment (the necessary volume is calculated based on the volume for one sample and total sample amount).

■ Sample pretreatment



Note:Use centrifuge tubes certified for high-speed centrifugation to ensure safe operation.

Test samples -Non-cellular samples

Non-cellular samples

①

Concentration

• Cell culture supernatant for 5% human albumin samples:
If sample volume ≤ 400 µL,concentration is not necessary;
If sample volume > 400 µL,centrifuge at 18000 ×g for 30 min,
remove the supernatant leave the remaining volume at around 400 µL.
• High-concentration plasmids:
Centrifuge at 18000 ×g for 30 min.
Remove the supernatant leave the remaining volume at around 100 µL.
Add 300 µL of Dilution buffer.

②

Sample pretreatment

Add Pretreatment buffer to the sample
(volume ratio 1:10),
vortex and mix well,
incubate at RT for 5 min.

③

Sample digestion

10 µL 5M NaCl
10 µL IC
20 µL Proteinase K
100 µL of Lysis buffer
55℃ for 60 min
（except for 5% human albumin samples at 25℃ for 60 min）

④

Test samples - Cell culture supernatant samples

Cell culture
supernatant samples

①

Cell removal

Centrifuge for 5 min at 72 ×g
transfer the supernatant to
a new centrifuge tube.

②

Concentration

If sample volume ≤ 400 µL,concentration is not necessary;
If sample volume > 400 µL,centrifuge at 18000 ×g for 30 min,
remove the supernatant leave the remaining volume at around 400 µL.

③

Sample pretreatment

Add Pretreatment buffer to the sample
(volume ratio 1:10),
vortex and mix well,
incubate at RT for 5 min.

④

Sample digestion

10 µL 5M NaCl
10 µL IC
20 µL Proteinase K
100 µL of Lysis buffer
55 ℃ for 60 min
（except for 5% human albumin samples at 25℃ for 60 min）

⑤

Note:Use centrifuge tubes certified for high-speed centrifugation to ensure safe operation.

For test samples (Please choose the appropriate procedure accordingly.)

- **Cell culture samples (≤ 10^7 cell/mL)**

1) Heat treatment

Incubate the samples at 95°C for 10 min and then cool the samples to 2-8°C for 20 min.

2) Sample pretreatment

After heat treatment, add Pretreatment buffer (NND002) to the sample at a volume ratio of 1:10, then vortex and mix well. Incubate at room temperature for 5 min.

3) Debris removal

Centrifuge at 7250×g for 10 seconds to precipitate all cell debris. Transfer the supernatant to a new tube.

4) Sample concentration

Centrifuge the supernatant in a refrigerated centrifuge at 18,000×g for 30 min. Remove the supernatant and leave the remaining volume at around 100-150 μL. Add 300 μL of Dilution buffer (NND022), vortex and mix well, then centrifuge in a microcentrifuge for 3 seconds.

- **Non-cellular samples**

1. High-concentration plasmids

1) Sample concentration

Centrifuge the sample in refrigerated centrifuge at 18,000×g for 30 min. Remove the supernatant and leave the remaining volume at around 100 μL. Add 300 μL of Dilution buffer (NND022).

2) Sample pretreatment

Add Pretreatment Buffer (NND002) to the sample at a volume ratio of 1:10, then vortex and mix well. Incubate at room temperature for 5 min, and centrifuge in microcentrifuge for 3 seconds.

2. 5% human albumin samples

1) Sample concentration

If the sample volume is ≤ 400 μL, concentration is not necessary. If the sample volume is > 400 μL, centrifuge the sample at 18,000×g for 30 min in a refrigerated centrifuge. Remove the supernatant with a pipette keeping a leftover that is less than 400 μL.

2) Sample pretreatment

Add Pretreatment buffer (NND002) to the sample at a volume ratio of 1:10. Vortex and mix well. Incubate at room temperature for 5 min, then centrifuge in a microcentrifuge for 3 seconds.

- **Cell culture supernatant samples**

1) Cell removal

Centrifuge at 72×g for 5 min to precipitate the cells. After centrifugation, transfer the supernatant to a new centrifuge tube.

2) Sample concentration

If the sample volume is ≤ 400 μL, concentration is not necessary. If the sample volume is > 400 μL, centrifuge the sample at 18,000×g for 30 min in a refrigerated centrifuge. Remove the supernatant with a pipette keeping a leftover that is less than 400 μL.

3) Sample pretreatment

Add Pretreatment buffer (NND002) to the sample at a volume ratio of 1:10. Vortex and mix well. Incubate at room temperature for 5 min, then centrifuge in a microcentrifuge for 3 seconds.

For control samples:

- **Negative control samples (NCS)**

NCS evaluates whether there is cross-contamination or environmental contamination during sample extraction.

Add 100-400μL of Dilution buffer (NND022) (use the same volume of Dilution buffer as the test sample) to the Pretreatment buffer (NND002) (Dilution buffer to Pretreatment buffer at a volume ratio of 10:1). Vortex to mix well and incubate at

room temperature for 5 min. Centrifuge 3 seconds in a microcentrifuge.

Note: If the sample matrix is added, follow the corresponding matrix pretreatment method in the procedure for test sample.

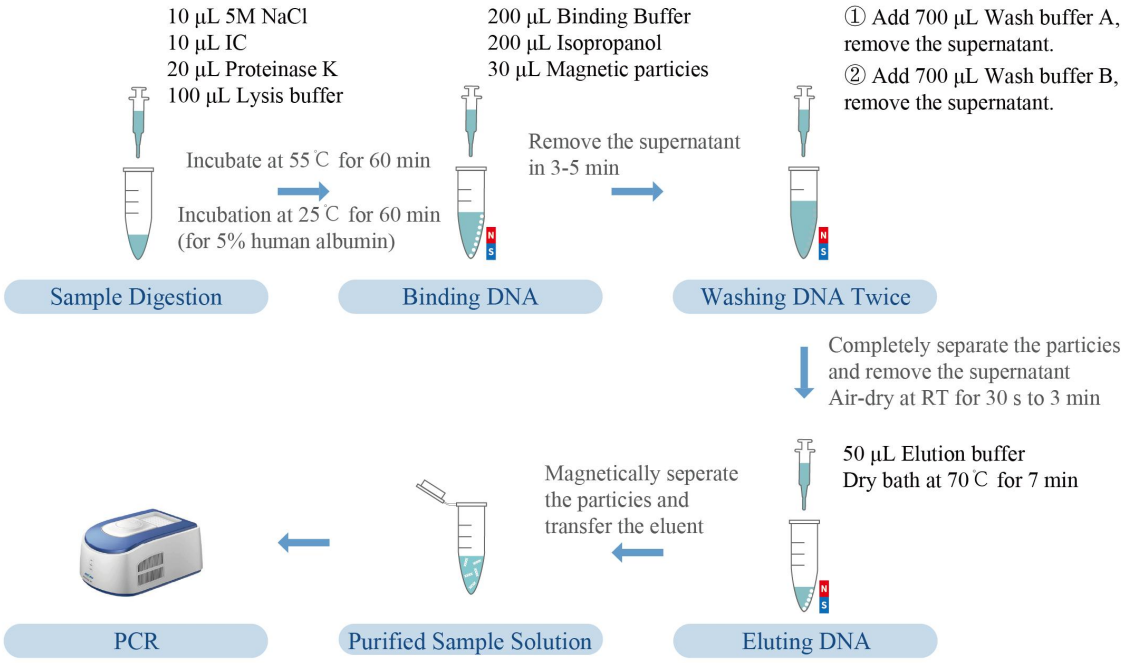
■ Samples digestion

1. To each sample, add 10 µL of 5M NaCl (NND040) and 10 µL of IC. Vortex to mix well, then centrifuge for 3 seconds in a microcentrifuge.
2. To each sample, add 20 µL of Proteinase K (NND023) and 100 µL of Lysis buffer (NND028). Vortex to mix well and then centrifuge for 3 seconds in a microcentrifuge. Incubate all samples, including the control samples at 55°C for 60 min, except for 5% human albumin samples and their corresponding control samples at 25°C for 60 min.

Note: For complete digestion, vortex to mix well after approximately 30 min of incubation, then continue incubation. After sample digestion, the following DNA extraction experiment should be performed as soon as possible.

■ Mycoplasma DNA extraction

1. manual extraction



Binding DNA

For each sample tube:

1) Incubate the Magnetic particles (NND033) at room temperature for 10 min, and vortex to mix well before use.
2) Remove the sample from the dry bath, and centrifuge it for 3 seconds, and add 200 μL Binding Buffer, vortex to mix well and centrifuge it for 3 seconds.
3) Add 200 μL of Isopropanol and 30 μL of Magnetic particles. Vortex the tubes vertically at medium speed for 5 minutes to bind the nucleic acids. Centrifuge for 10 seconds. Place the tubes on the magnetic stand with Magnetic particles pellet facing the magnet.
4) Wait until the solution is clear and the particles are fully separated (about 3-5 minutes). Carefully remove the supernatant without disturbing the magnetic particles.

Washing DNA

For each tube with magnetic particles (binding nucleic acids):

1) Add 700 μL of Wash buffer A. Vortex for 10 seconds and centrifuge for 10 seconds, then place the tubes in the magnetic stand. Carefully remove the supernatant without disturbing the magnetic particles to complete the first washing.
2) Add 700 μL of Wash buffer B. Vortex for 40 seconds and centrifuge for 10 seconds, then place the tubes in the magnetic stand. Carefully remove the supernatant without disturbing the magnetic particles to complete the second washing.
3) To remove supernatant completely, centrifuge in a microcentrifuge for 10 seconds and place each tube in the magnetic stand again. Wait until the particles completely separate, use a 10 μL volume pipette to carefully remove the residual supernatant.

Note:When removing the supernatant, avoid removing the magnetic particles together with the supernatant.

4) With the cap open, air-dry the magnetic particles pellet at room temperature for 30s - 3min to remove any residual ethanol.

Note: The drying time depends on the specific environment. It could be shorter in higher temperature or lower humidity condition, while slightly longer in lower temperature or higher humidity condition.

Eluting DNA

For each sample:

1) Add 50 μL Elution buffer (NND019) , vortex for 5 seconds and incubate at 70℃ for 7 min. Vortex 2-3 times during incubation to ensure complete resuspension of the magnetic particles.

Note: Vortex the Magnetic particles and Elution buffer thoroughly. If Magnetic particles and Elution buffer stick to the tube cap or wall, briefly centrifuge again.

2) After incubation, centrifuge for 1 min, and then place the tubes in the magnetic stand. Wait until the particles completely separate, carefully transfer the eluate to a new microcentrifuge tube.

3) Quickly centrifuge the tube for 10 seconds, and then place it in the magnetic stand again. Carefully transfer the eluate to a 1.5 -mL microcentrifuge tube to obtain the purified sample solution.

Note: Transfer the eluate completely and avoid leaving any residuals behind.

2. Automated procedure with rHCDpurify

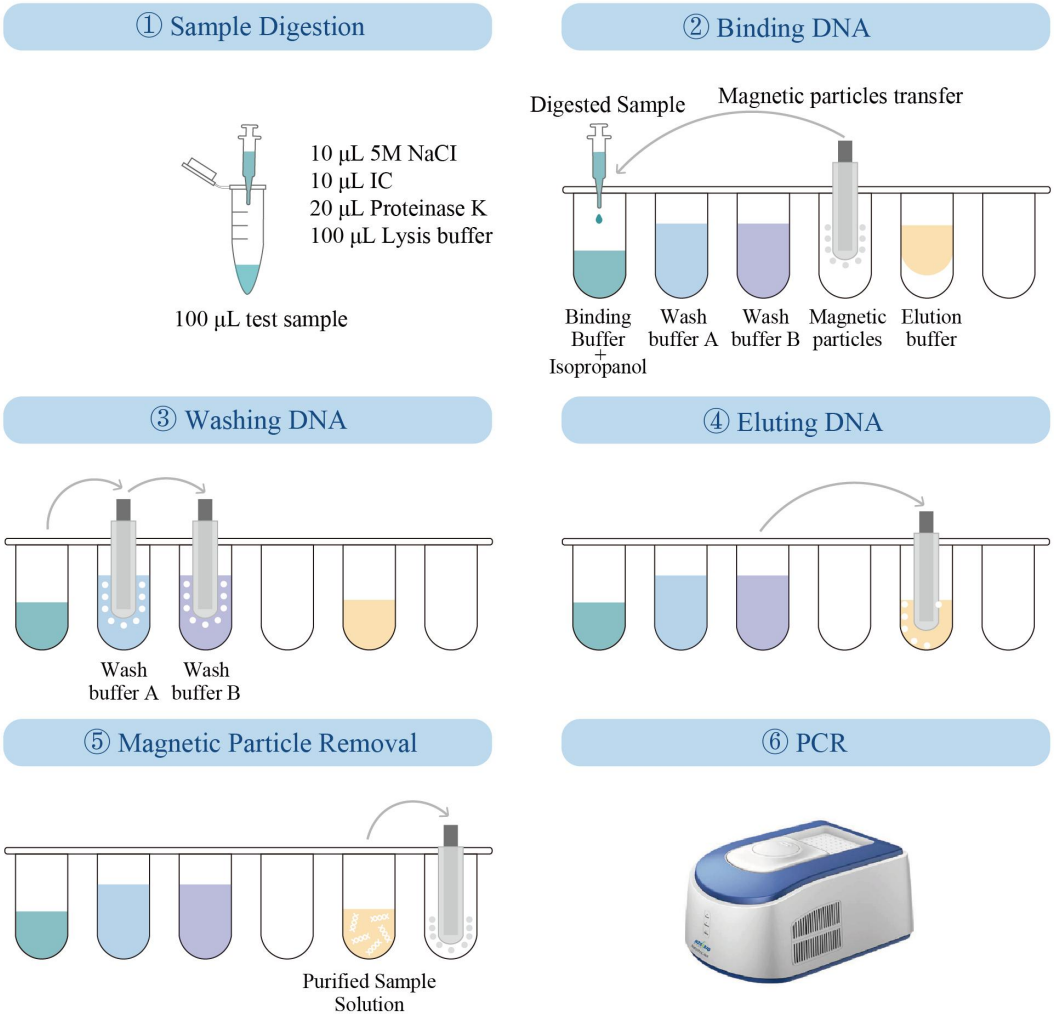


Plate preparation

Add the corresponding solution to each well according to the following 96 deep-well plate layout:

Table 2. Example of 96-well Plate layout

	Group 1					Group 2					
1	2	3	4	5	6	7	8	9	10	11	12
S1											
S2											
S3											
S4											
S5											
S6											
S7											
S8						NCS					

Column 1 or 7: Binding Buffer 200 µL/well, Isopropanol 200 µL/well

Column 2 or 8: Wash buffer A, 700 µL/well

Column 3 or 9: Wash buffer B, 700 µL/well

Column 4 or 10: Magnetic particles, 30 µL/well

Column 5 or 11: Elution buffer, 65 µL/well

Note:Add samples to column 1 or 7 after transferring all reagents.

Program Processing

Start the program before plate preparation:

Power on—self-test—  .

1. Use 75% ethanol wipes to clean the insider walls.

2.  —UV—  —  15min—  .

After plate is prepared, continue with the following steps:

3. Put the 96-deep well plate into the instrument and insert the plastic sleeve into the corresponding position.

4. Run—  —Myco-2G—  , run about 43 min.

5. After extraction, immediately take out the deep-well plate, and transfer each

purified sample solution to a 1.5mL microcentrifuge tube.

Precautions

1. *It is recommended to divide the laboratory into separate areas, including the negative area (for preparation of negative control samples, PCR reagents, and negative test controls), the positive area (for sample preparation), and the amplification area, etc. Each area should be clearly marked and equipped with independent sets of equipment and supplies to prevent cross-contamination. Experimental reagents, test samples, and PCR amplification products should be stored separately and not in the same storage location. Avoid unnecessary movement within the laboratory to reduce the risk of contamination.*
2. *Before starting the experiment, ensure that the ambient temperature is not lower than 22°C.*
3. *During the experiment, select appropriately sized gloves and change them regularly. Use different lab coats, masks, hair covers, and gloves in different experimental areas to avoid cross-contamination.*
4. *Centrifuge the tubes before opening to avoid contamination of gloves or pipettes. Handle tubes with care to prevent splashing.*
5. *Dispose of used tips and liquid waste according to the regulations.*
6. *After PCR amplification, wear disposable gloves to remove the PCR tubes or plates. Check whether the caps or seals are tightly closed and whether the walls are cracked. Discard in the designated area; do not open the caps.*
7. *During the process of separating magnetic particles using the magnetic stand, rotate the tubes slowly to accelerate the aggregation of magnetic particles.*
8. *During DNA washing and eluting, ensure that no magnetic particles or liquid remain on the tube caps or walls; if so, perform a brief centrifugation.*
9. *When removing residual ethanol, avoid over-drying, which may cause incomplete dissolution during the eluting process.*
10. *Please perform DNA detection on the same day after DNA extraction to ensure accuracy.*
11. *Before starting the rHCDpurify program, check whether the 96-well deep plate and the sleeve are properly secured.*

12. Before and after running the rHCDpurify program, UV sterilize the machine for at least 15 minutes and clean the inner walls with 75% ethanol wipes. The minimum interval between two extractions should be 30 minutes.

13. Immediately after the rHCDpurify program, transfer each sample solution to a new microcentrifuge tube. Condensation on the walls of the 5th or 11th row wells will not affect DNA extraction; simply transfer the bottom eluate to a new tube and ensure that more than 40 μL of eluate is collected for the assay.

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Support & Contact

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