Pathogen DNA/RNA Purification Kit (Beads)

(BW-MGD2411)

BEIWO Pathogen DNA/RNA Purification Kit is designed for the extraction and purification of pathogen DNA/RNA. The unique buffer solution system optimized for various samples such as blood, serum, plasma, urine, sputum, nasopharyngeal swab, nasopharyngeal aspirate, cerebrospinal fluid, pleural effusion, is suitable for the extraction of bacteria, fungal, viruses, etc. This kit is compatible with BEIWO BW Express 16, Allsheng Auto- Pure 32A and other similar nucleic acid purification systems.

KIT CONTENTS

Catalog#	BW-MGD2411-A00	BW-MGD2411-A32-32		BW-MGD2411-A32		BW-MGD2411-A96	
	Manual operation	Well position		Well position		Plate position	
Preps	50 T	1Tx32		1x32T		1x96T	
Lysis Buffer A	50mL	Well 1	600 µL	Column 1/7	600 µL	Plate 2	600 µL
MgPure Beads	1.25mL	Well 2	400 µL	Column 2/8	400 µL	Plate 3	400 µL
Wash Buffer 1	50mL	Well 3	600 µL	Column 3/9	600 µL	Plate 4	600 µL
Wash Buffer 2	100mL	Well 4	800 µL	Column 4/10	800 µL	Plate 5	800 µL
Wash Buffer 3	-	Well 5	800ul	Column 5/11	800ul	Plate 6	800µL
DEPC-Treated ddH2O	10mL	Well 6	80 µL	Column 6/12	80 µL	Plate 8	80 µL
Proteinase K	1.25mL		800 uL		800 uL		2x1.25mL
Tip Comb	-		8		4		1

*BW-MGD2411-A32 and BW-MGD2411-A32-32 are 8 strip tip comb, BW-MGD2411-A96 is a 96-well Tip Comb.

STORAGE

The kit should be stored dry and at room temperature (15~25°C). They can be stored for at least 12 months without showing any significant reduction in performance, capacity, or quality of separation.

BEFORE STARTING

- Operate in an environment with the appropriate biosafety laboratory level (e.g BSL-2 or higher level) and wear appropriate personal protective equipment (e.g. gowns, gloves, goggles) when working with clinical specimens.
- Lysis Buffer A contains chaotropic salts, which may form reactive compounds when combines with bleach. Do not add bleach or acidic solutions directly to the preparation waste.
- Lysis Buffer A may form precipitates upon storage, dissolve precipitates at 37℃ before use.
- Ultraviolet disinfection of the Purification Instrument prior to use is recommended.
- Materials not supplied: 1.5 mL RNase-free centrifuge tube.
- Materials not supplied: Metal rack（Cat No. BW-CB137）,it can be reused.
- RNase A is not included in the kit and needs to be purchased separately (Cat No.BW-B0052).
- Lysozyme is not included in the kit and needs to be purchased separately (Cat No.BW-B003).

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.
- ✓ The product should be used in strict accordance with the instructions. If it is not used in accordance with the regulations, it may cause pollution to the environment.
- ✓ Products are disposable, do not reuse, and the used product should be placed in the designated position in time.
- ✓ Exceeding the expiration date of this product, the performance of the product may be reduced, so it should be used within the expiration date.
- ✓ This kit can only be used for in vitro experiments, and not for clinical, therapeutic and in vivo experiments in animals. If it is not used in accordance with the regulations, the company will not be responsible for the consequences arising therefrom.

Sample Processing

Cerebrospinal fluid, alveolar lavage fluid, and sputum samples:

1. 700 μL~800 μL samples were added to 2.0 mL screw tubes (500 μL zirconia beads or glass beads were added), put into a wall breaker, break the wall at 5000 rpm for 1 min, centrifuge at 8000 rpm for 1 min.
2. 200 μL of the supernatant sample was added to a 1.5 mL centrifuge tube for further operation.

Mucous sputum samples:

1. Add 500 μL of 2% NaOH solution (Reagent kit not available) to 500 μL of the sample, tightly cap the tube, shake and mix for 1 min, and let stand at room temperature for 15-20 min, not more than 20 min, until the sputum is completely fluid.
2. Centrifuge at 10,000 rpm for 1 min.
3. Aspirate the supernatant, add 200 μL of PBS Buffer or molecular grade water to the bottom of the reselected tube and continue the procedure.

★Preparation of 2% NaOH solution: Take 1 g of NaOH, dissolve in 40 mL of purified water, and fix the volume to 50 mL after dissolution.

Microbial cultures:

a. Gram-negative cultures:

1. Take 1 mL of culture in a 1.5 mL centrifuge tube at 8,000 rpm, centrifuge for 5 min, discard supernatant
2. 800 μL of the supernatant was discarded, and 200 μL was retained for resuspension of the microbial cells by shaking and continued operation.

b. Gram-positive cultures:

1. Take 1 mL of bacterial solution in a 1.5 mL centrifuge tube, centrifuge at 8,000 rpm for 5 min, discard supernatant 900 μL and retain 100 μL, 100 μL of lysozyme (40 mg/mL, reagent kit not available)) was added and incubated at 37°C for 20 min.
2. Incubate for 20 min at 37°C and continue operation.

EXTRACTION PURIFICATION PROCEDURE

Manual operation (BW-MGD2411-A00)

1. The Lysis Tubes were added with 200~400μL sample, 600μL **Lysis Buffer A** and 25uL**proteinase K** at 55℃, and max vortex for 15 min. Optional: If RNA free gDNA is desired, add 5 μL RNase A to the Lysis Buffer A.

2. Add 20 μL **MgPure Beads** into the 1.5mL tube at room temperature and votex for 5 min.

Note: The MgPure Beads should be vortex to ensure full suspension before use.

3. After the 1.5mL tube is immediately separated and no liquid remains on the tube wall is ensured, the 1.5mL tube is placed on the magnetic stand for 2 min or until the MgPure Beads are completely absorbed, and all supernatants are carefully absorbed and discarded with a pipette.

4. Add 600 μL **Wash Buffer 1** into the 1.5mL tube, blow with pipette 5-10 times, place the 1.5mL tube on the magnetic rack for 2 min or until the MgPure Beads are completely absorbed, and carefully absorb and discard all supernatant with pipette.

5. Add 800 μL **Wash Buffer 2** into the 1.5mL tube, blow with pipette 5-10 times, place the 1.5mL tube on the magnetic rack for 2 min or until the MgPure Beads are completely absorbed, and carefully absorb and discard all supernatant with pipette.

6. Repeat step 5.

7. The 1.5mL tube was placed on the magnetic rack and left open to dry for 5 min.

8. Add 80 μL **DEPC-Treated ddH2O** into the 1.5mL tube, blow and mix with pipette, and incubate at 56℃ for 5 min. After the 1.5mL tube is transient, the 1.5mL tube is placed on the magnetic rack for 2 min or until the magnetic bead is completely absorbed, and all supernatant is transferred to the new centrifuge tube with a pipette. The obtained nucleic acid solution was stored at -20℃ for a long time

Nucleic acid extractor（BEIWO BW Express 16 or an Allsheng Auto-Pure 32A）（**BW-MGD2411-A32-32**）

1. Take out a pre-loaded 6-well strip and gently shake it (if necessary), let the reagent or magnetic beads assemble at the bottom of the well.

NOTE: If there is any precipitation in **Well 1**, incubate the plate at 37℃ to dissolve the precipitation before use.

2. Place the 6-well strip into Metal rack for the automated extractor in the correct orientation according to its shape; remove the sealing film carefully and avoid violent shaking to prevent spilling of liquid.

3. In a biosafety cabinet, carefully remove the sealing foil of the 6-well strip. Aspirate **200~400 uL** samples and **25 uL proteinase K** into the deep wells in Well 1. Optional: If RNA free gDNA is desired, add **5μL** RNase A to the Lysis Buffer A.
4. Put the Strip in an Allsheng Auto-Pure 32A Nucleic Acid Purification System.
5. Install two 8-strip Tip Combs for each Rack.
6. Run the program described in Table 1.
7. After the program is completed, take out the 6-well strip and transfer the eluate to a new sterile tube of choice for final storage.

Table 1. Recommended program for Auto-Pure 32A Nucleic Acid Purification System

Step	Well	Name	Mix time (min)	Magnet (sec)	Wait time (min)	Vol. (μL)	Mix speed (1- 10)	Temp. (℃)	Mix pos (0- 100%)	Mix amp (1- 100%)	Magnet pos (0- 100%)	Magnet speed (1- 10)
1	1	Lysis	15	0	0	1000	10	55	0	80	0	1
2	2	Beads	0.5	60	0	800	8	OFF	0	80	0	1
3	1	Bind	5	40	0	800	9	100	0	80	0	1
4	3	Wash1	1	20	0	600	9	OFF	0	80	0	1
5	4	Wash2	1	20	0	800	9	OFF	0	80	0	1
6	5	Wash3	1	20	1	800	9	OFF	0	80	0	1
7	6	Elute	5	40	0	80	10	90	0	80	0	1
8	4	Drop	0.5	0	0	800	8	OFF	0	80	0	1

Note: Set 'Heating synchronization', 'Cool Fan Disabled', 'Cooling synchronization', and 'Drying Fan Disabled'

Nucleic acid extractor（BEIWO BW Express 16 or an Allsheng Auto-Pure 32A）(BW-MGD2411-A32)

1. If necessary, gently shake the pre-loaded 96-well plate to let the reagent or magnetic beads assemble at the bottom of the plate.

NOTE: If there is any precipitation in **Column 1/7**, incubate the plate at 37°C to dissolve the precipitation before use.

2. Carefully remove the sealing foil of the 96-well plate, aspirate**200~400 μL sample** and **25 μL proteinase K** into the deep well in **Column 1/7**. **Optional:** If RNA free gDNA is desired, add **5 μL RNase A** into each well in **Column 1/7**.
3. Put the plate in a BEIWO BW Express 16 or an Allsheng Auto-Pure 32A Nucleic Acid Purification System.
4. Install two 8-strip Tip Combs for each plate.
5. Run the program described in Table 2.
6. After the program is completed, take out the 96-well plate and transfer the eluate into new sterile tube of choice for final storage.

Table 2. Recommended program for Auto-Pure 32A Nucleic Acid Purification System

Step	Well	Name	Mix time (min)	Magnet (sec)	Wait time (min)	Vol. (μL)	Mix speed (1-10)	Temp. (℃)	Mix pos (0-100%)	Mix amp (1-100%)	Magnet pos (0-100%)	Magnet speed (1-10)
1	1	Lysis	15	0	0	1000	10	55	0	80	0	1
2	2	Beads	0.5	10	0	400	10	OFF	0	80	0	1
3	1	Bind	5	20	0	1000	10	OFF	0	80	0	1
4	3	Wash1	1	10	0	600	10	OFF	0	80	0	1
5	4	Wash2	1	10	0	800	10	OFF	0	80	0	1
6	5	Wash3	1	10	1	800	10	OFF	0	80	0	1
7	6	Elute	5	20	0	100	10	85	0	80	0	1
8	2	Drop	0.5	0	0	400	5	OFF	0	80	0	1

Note: Set 'Heating synchronization', 'Cool Fan Disabled', 'Cooling synchronization', and 'Drying Fan Disabled'

Nucleic acid extractor（Allsheng Auto-Pure 96A） (BW-MGD2411-A96)

1. Take out the 96-well plates required for single batch gDNA extraction. Gently shake the plates if necessary to let the reagent or magnetic beads assemble at the bottom of the plates.

NOTE: If there is any precipitation in **Lysis Buffer A**, incubate the plate at 37°C to dissolve the precipitation before use.

2. Carefully remove the sealing foil of the 96-well plate named Lysis Buffer A, aspirate **200~400 μL sample** and **25 μL proteinase K** into the deep well. Optional: If RNA free gDNA is desired, add 5 μL RNase A to the Lysis Buffer A.
3. Put the Lysis Buffer A plate on position 2 of the Allsheng Auto-Pure 96 instrument.
4. Carefully remove the sealing foil of the 96-well plate named Wash Buffer 3, put a 96-Well Tip Comb into the plate, and put them on position 6 of the Auto-Pure 96 instrument together.
5. Carefully remove the sealing foils of other 96-well plates and put them on the corresponding positions according to the position specified in the KIT CONTENTS table as well as marked on the plate labels.
6. Run the program described in Table 3.
7. After the program is completed, take out the 96-well plate and transfer the eluate into new sterile tube of choice for final storage.

Table3. Recommended program for Auto-Pure 96 Nucleic Acid Purification System

Step	Name	Plate	Mix Time (min)	Mix Amp (%)	Wait Time (min)	Vol. (μL)	Mix Speed (1-10)	Temp. (℃)	Seg-ments (0-5)	1st Seg. time (s)	2nd Seg. time (s)	3rd Seg. time (s)	4th Seg. time (s)	5th Seg. time (s)	Cycle times (1-10)	Mag. speed (1-10)	Lip-lvl (0-30s)	Anti-Splash (0-30s)
1	Load	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2	Lysis	2	15	80	0	1000	9	55	0	-	-	-	-	-	-	-	0	0
3	Beads	3	0.3	80	0	400	4	OFF	1	10	-	-	-	-	1	1	0	0
4	Bind	2	5	80	0	1000	10	OFF	2	10	10	-	-	-	1	1	0	0
5	Wash1	4	1	80	0	600	10	OFF	1	10	-	-	-	-	1	1	0	0
6	Wash2	5	1	80	0	800	10	OFF	1	10	-	-	-	-	1	1	0	0
7	Wash3	6	1	80	1	800	10	OFF	1	10	-	-	-	-	1	1	0	0
8	Elute	8	5	80	0	100	3	85	1	20	-	-	-	-	1	1	0	0
9	Unload	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Note: Set 'Heating synchronization', 'Cool Fan Disabled', 'Cooling synchronization', and 'Drying Fan Disabled'

LIMITED USE AND WARRANTY

This product is warranted to perform as described in its labeling and in BEIWO's literature when used in accordance with instructions. No other warranties of any kind, express or implied, including, without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by BEIWO. BEIWO's sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of BEIWO, to replace the products, BEIWO shall have no liability for any direct, indirect, consequential, or incidental damage arising out of the use, the results of use, or the inability to use it product. For technical support or learn more product information, please contact us at 400-115-2855 or visit our website at www.beiwobiomedical.com

Labels, Packing Logo Design

Symbol	Introductions	Symbol	Introductions
LOT	Batch Code		Consult instructions for use
IVD	For in vitro diagnostic device use		Manufacture Date
	Manufacturer Name Address	EC REP	Name and Address of European Union Representative
CE	CE Symbol		Used-by date
REF	Catalogue Number		Importer
	Distributor	UDI	Unique Device Identification
CC	Country of Manufacture	#	Model Number
	Do not reuse" are "single use, "Use only once		

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