Virus DNA/RNA Extraction Kit

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【Product Name】

Virus DNA/RNA Extraction Kit

【Product Model】

HMD3502，HMD3602-16T，HMD3602-96T.

【Packing Specification】

100T/kits, 16T/plate, 96T/box.

【Intended Use】

For nucleic acid extraction, enrichment, purification and other steps. The treated products are used for clinical in vitro detection.

【Inspection Principle】

This product utilizes the specific adsorption capacity of magnetic beads for nucleic acid in the environment of high ionization salt to enrich nucleic acid in the sample lysates. After washing for removing impurities, the enriched nucleic acid is released in the eluate to achieve the purpose of nucleic acid purification.

【Main components】

1. Each functional component of HMD3602-16T and HMD3602-96T has been packed in deep hole plate in preloading, Lysis buffer 450 μL/test, Wash buffer 1500μL and Wash buffer 2700 μL×2 test, Magnetic beads 200 μL/test, and Eluate 50 μL/test.
2. HMD3502 put the corresponding amount of Lysis buffer (450 μL/test), Wash buffer 1 (500 μL/test), and Wash buffer 2 (1400 μL/test). Eluent (50 μL/test), Magnetic beads (200 μL/test) in different containers, and separate them into deep well plate or other container by the operator before use.
3. Reagent specifications:

Plate packaging:

Table 1 Main components of HMD3602-16T

	component			**Specification and Quantity**
96-well deep-hole plate pre-loaded nucleic acid extraction reagent	Column	Volume	Solution name	HMD3602-16T
	1 or 7	450 μL	Lysis buffer	1
	2 or 8	200 μL	Magnetic beads	
	3 or 9	500 μL	Wash buffer 1	

	4 or 10	700 μL	Wash buffer 2	
	5 or 11	700 μL	Wash buffer 2	
	6 or 12	50 μL	Eluent	
	8 magnetic stirring sleeve			2

Table 2 Main components of HMD3602-96T

	component			**Specification and Quantity**
96-well deep-hole plate pre-loaded nucleic acid extraction reagent	Plate	Volume	Solution name	HMD3602-96T
	1	450 μL	Lysis buffer	One for each, 6 pieces in total
	2	200 μL	Magnetic beads	
	3	500 μL	Wash buffer 1	
	4	700 μL	Wash buffer 2	
	5	700 μL	Wash buffer 2	
	6	50 μL	Eluent	
	96-Well magnetic stirring sleeve			1

Bottle packaging:

Table 3 Main components of HMD3502

Kit Contents	**HMD3502 100T**	**Main components**
Lysis Buffer	＞45 mL	Guanidine salt，Tris-HCl
Magnetic Beads	＞20 mL	Nano magnetic beads
Wash buffer 1	＞50 mL	Guanidine salt，Tris-HCl
Wash buffer 2	＞140 mL	Ethanol
Eluent	＞6 mL	Ultrapure water

【Storage Condition and Validation Date】

Keep storage at 2℃-25℃. Freezing can greatly reduce the performance of magnetic beads. Please avoid freezing during storage and transportation. This reagent is valid for 12 months under appropriate storage. Use the pre-loaded reagent as soon as possible after opening it.

【Applicable Instruments】

Allsheng Auto-Pure 32A automatic nucleic acid extractor and similar instruments.

【Sample Requirements】

1. This product is suitable for nucleic acid extraction of cells and pathogens from nasopharyngeal swabs, saliva, urine, plasma, serum, cell preservation fluid and other liquid samples.
2. Immediately after collection, samples should be placed into cell preservation solution that can be used for nucleic acid detection for fixation and preservation, and nucleic acid extraction should be carried out as soon as possible.
3. Nuclease can affect the performance of this product
4. Saliva sample: take more than 200μL saliva sample, if less than 200μL, please use all.
5. Dry swab sample: add 400μL PBS (sample storage solution with sample swab) to sample tube with swab head, vortex for 2min, then use 200μL supernatant after centrifugal at top speed.
6. Wet swab sample: mix with high-speed vortex for 1min, absorb 200μL for use (if the liquid is less than 200μL, add appropriate amount of PBS or sample storage solution with the sampling swab, mix with vortex and centrifuge).
7. Other liquid samples: more than 200μL take 200μL, if less than 200μL, please use all.

【Self-provided Materials】

1. Automatic nucleic acid extraction instruction (For automatic extraction methods, this description takes HMD3602-16T and Allsheng Auto-Pure32A automatic nucleic acid extraction instrument as an example.
2. 96-well deep hole plate and magnetic rod sleeve (HMD3502 automatic extraction).
3. Magnetic rack (HMD3502 manual extraction).

【Preparations】

1. Please take out the buffer from the refrigerator, and use it till room temperature.
2. If there is a small amount of precipitation in the lysis buffer, please place it at room temperature or 37℃ and use it after the precipitation is fully dissolved.
3. Before use, magnetic beads must be shaken until there is no visible precipitation at the bottom before being used.

【Operation Steps】

HMD3602-16T/HMD3602-96T automatic extraction

1. Unseal: take out the deep-hole plate from the packaging box, balance to room temperature, carefully tear off the sealing film, if there is liquid adhesion to the deep-hole plate sealing film and tube wall, throw the liquid to the bottom and then unseal.
2. Add sample: Add 200 μL sample to column 1/7 (HMD3602-16T) or plate 1 (HMD3602-96T), one sample for each well position. Note: please add sample within 20 minutes after opening.
3. Load in machine: set extraction procedures according to supplemental table. Put the deep-hole plate into the nucleic acid extraction instrument, insert the magnetic rod sleeve into the corresponding slot, and start the program. After the procedure was completed, the eluent was transferred to a centrifuge tube and the product was stored at -80°C. Discard waste according to standard procedures.

HMD3502 manual extraction

1. Add 200 μL samples and 450 μL lysis buffer into a 1.5 mL centrifugation tube, mix them upside down, and incubate at 55°C for 5 minutes.

2. Add 200μL magnetic beads, rotate and incubate at room temperature on a rotating blender for 10 minutes.
3. Place the mixture containing the sample on the magnetic rack and let stand for 2 minutes (or until the lysis buffer is colorless and transparent). Suck out liquid phase and discard. Note: If there are magnetic beads on the cover of the centrifugation tube, the magnetic beads can be washed off by reversing the centrifugation tube with the magnetic rack 3-4 times during the standing process.
4. Remove the magnetic rack and add 500 μL wash buffer 1. Mix upside down until the beads are fully suspended or blow the beads 3-4 times with a pipette.
5. Place the centrifugal tube on the magnetic rack, stand for 1 minute and discard the supernatant.
6. Remove the magnetic rack, add 700 μL wash buffer 2, and re-suspend the magnetic beads.
7. Place the centrifugation tube on the magnetic rack, stand for 1 minute and discard the supernatant.
8. Repeat Steps 6 and 7. Note: Try to ensure that the residual liquid in the centrifugation tube is completely removed (including the residual liquid on the cover of the centrifugation tube).
9. Keep the centrifugation tube on the magnetic rack, open the cover and let stand at room temperature to dry for 5 minutes.
10. Remove the magnetic rack, add 50μL eluent, and re-suspend the magnetic beads. Incubate at 55℃ for 5 min, and gently flick the centrifugation tube 2-3 times during the incubation period to separate the magnetic beads Scattered.
11. Place the centrifugation tube on the magnetic rack and let stand for 2 minutes. Transfer the supernatant containing elution nucleic acid into the new centrifugation tube. Note: If there is liquid attached to the wall of the centrifugation tube, please immediately centrifugation before magnetic suction operation.
12. Discard the experimental waste liquid as required, and store the purified nucleic acid samples in appropriate conditions or conduct the next experiment.

HMD3502 automatic extraction

1. Add samples: add samples in the order of Table 4.

Table 4. HMD3502 automatic extraction and sampling sequence

96 wells Plate	Orders and Dosages
1 or 7	1. 450μL lysis buffer 2. 200μL sample
2 or 8	3. 200μL magnetic beads
3 or 9	4.500μL Wash buffer 1
4 or 10	5.700μL Wash buffer 2
5 or 11	6.700μL Wash buffer 2
6 or 12	7. 50μL Eluent

2. Load in machine: set extraction procedures in accordance with supplemental table. Put the deep-hole plate into the nucleic acid extraction instrument, insert the magnetic rod sleeve into the corresponding slot, and start the program. After the procedure was completed, the eluent in well 6 or 12 was transferred to a centrifuge tube and the product was stored at -80℃. Discard waste according to standard procedures.

【Limitations of test methods】

Automatic extraction of this product needs to be used together with automatic nucleic acid extraction instrument. Manual extraction should be used together with magnetic rack.

【Precautions】

1. This product is only for in vitro diagnostic use, not for human or animal internal and external use.
2. The extracted nucleic acid should be used for testing as soon as possible. If it is not tested immediately, the nucleic acid should be removed from the deep hole plate, placed in a container without nuclease, and stored at -20℃.
3. During the operation, please wear disposable gloves, disposable masks and lab coats. Nuclease-free consumables are used to minimize RNase contamination. The lysis buffer and Wash buffer 1 contain guanidine isothiocyanate or guanidine hydrochloride, which will cause corrosion to skin and mucous membrane, If it is not carefully exposed to eyes, Please rinse with plenty of water and seek medical advice.
4. Do not mix waste liquid with bleach or acidic solution to avoid toxic volatile substances.

【Performance】

1. Reagent packaging should be intact and tidy.
2. Net content of each component: no less than marked amount.
3. Extraction yield: Nucleic acid purification for 100 μL~400 μL liquid sample, eluent yield is not less than 42 μL.
4. Nucleic acid recovery rate: nucleic acid purification is performed on the calibrated DNA samples, and the recovery rate is no less than 90%.
5. Purity of nucleic acid: Nucleic acid purification was carried out on the calibrated DNA sample, and the OD_{260}/OD_{280} product was between 1.7 - 2.0.
6. In-batch precision: Ct value is obtained by amplification of extract using calibrated reference and fluorescent PCR reagent specified by the enterprise, with a repeatability CV of no more than 5%.
7. The purified nucleic acid can be detected by PCR and other molecular biology.

Eg：Extract 200μL with density of 5×103 IU/mL COVID-19 pseudovirus throat swab sample, test with COVID-19 detection kit. After 10 parallel experiments, results shown that detection rate is 100% and reproducibility of Ct value is good (Figure 1). RT-qPCR detection is performed on the extracted products from pharyngeal swabs of different concentrations of COVID-19 pseudovirus, and the results show that the extraction ability of samples of different concentrations of COVID-19(5×10^1 IU/mL，5×10^2 IU/mL，5×103 IU/mL，5×10^4 IU/mL，5×10^5 IU/mL) is good and the differentiation degree is obvious (Figure 2). The logarithm of Ct value of RT-qPCR and the number of pseudovirus is analyzed by linear regression. The results showed well linearity (Figure 3).

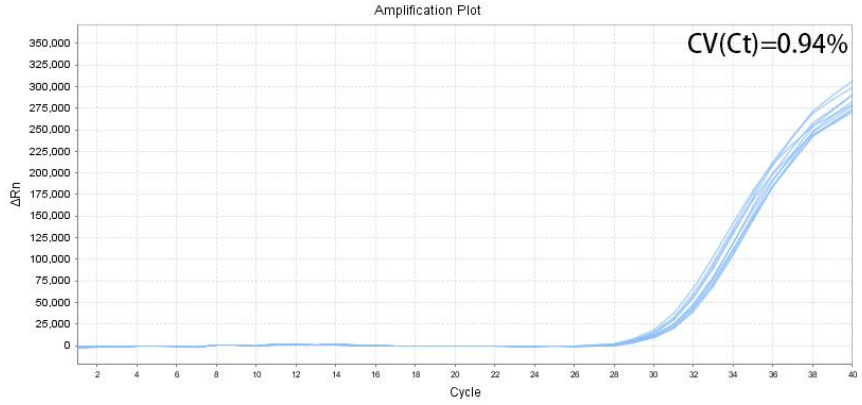

Figure 1. COVID19 pseudovirus (5000IU/mL) amplification curve of throat swab sample

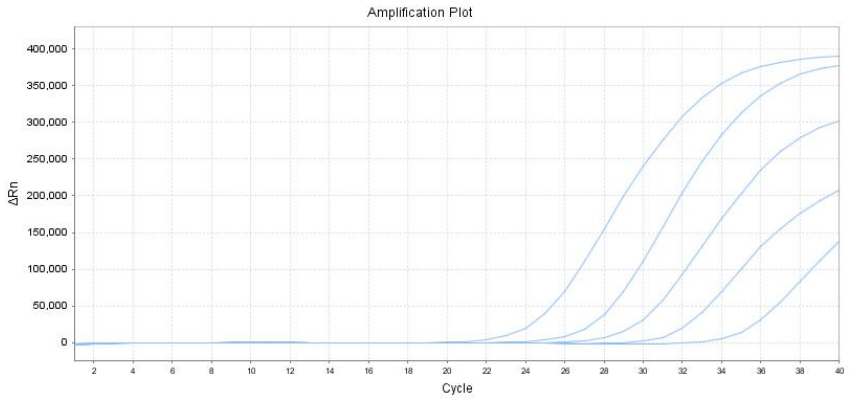

Figure 2. COVID19 amplification curve of pseudovirus gradient sample

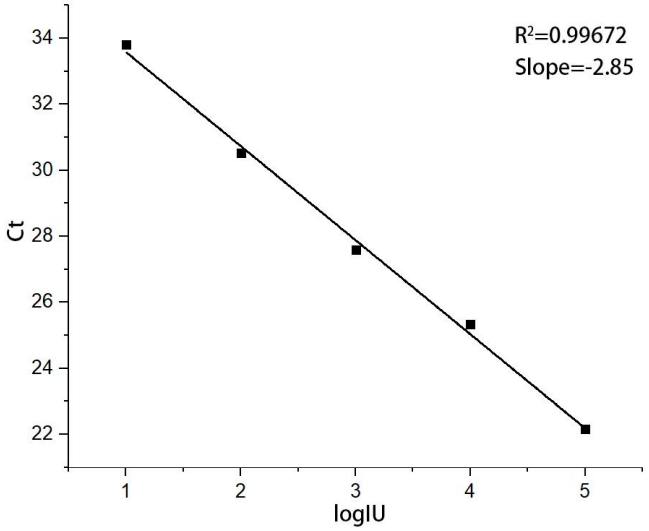

Figure 3. Nucleic acid extraction and detection results is fitted linearly

【References】

He H, Li R, Chen Y, et al. Integrated DNA and RNA extraction using magnetic beads from viral pathogens causing acute respiratory infections. Sci Rep. 2017; 7: 45199.

Saiyed ZM, Ramchand CN, Telang SD. Isolation of genomic DNA using magnetic nanoparticles as a solid-phase support. J Phys Condens Matter. 2008; 20(20):204153.

步骤	孔位	名称	混合时间（min）	磁吸时间（sec）	等待时间（min）	容积（μL）	混合速度	温度（℃）	混合位置	混合幅度	磁吸位置	磁吸速度
1	2	Transfer	0	20	0.0	200	10	0	0	80	10	10
2	1	Lysis	3.0	0	0.0	650	10	55	0	80	10	1
3	1	Binding	3.0	20	0.0	650	10	0	0	80	10	10
4	3	Wash1	1	20	0.0	500	8	0	0	80	10	10
5	4	Wash2	0.5	20	0.0	700	8	0	0	80	10	10
6	5	Wash2	1	20	4.0	700	8	0	0	80	10	10
7	6	Elute	3.0	30	0.0	50	10	70	0	80	10	10