Version: FML1812

DNA/RNA Extraction Kit (Magnetic Beads)

[Packaging] 202-Y：10serving/box, 16serving/box,32serving/box,40serving/box,64serving/box, 96serving/box. 202-B：50serving/box, 96serving/box, 960serving/box.

[Intended Use] It is suitable for separating and purifying high-quality genomic DNA from a variety of plant tissues.

[Principle] Nucleic acids DNA from a complex with magnetic beads in a specially formulated buffer. The beads nucleic acids complex is then separated from lysates using a magnet. Purified DNA are then eluted when the buffer condition is adjusted. Special magnetic beads technology enables purification of high-quality nucleic acids that are free of proteins, nucleases, and other impurities.

[kit Contents] Composed of lysis, eluent and magnetic beads, etc.

[Storage]All Reagents can be stored at room temperature (15–25°C) for 12 months.

[**Sample Requirements**] Blood、saliva、swabs、Animal tissue、bacteria、cells etc.

[Protocol]

Table 1. Product Application

Type	M202	Y202-G10 / Y202-G10-S	Y202-G30
Applicable method	Manual Operation	Automated purification (for GENFINE Purifier 32)	Automated purification (for GENFINE P96)

Part 1：Sample pretreatment

A. Blood（Anticoagulant）

1. Take 250 µl blood sample into a 1.5 ml centrifuge tube.

2. Add 20 µl Proteinase K solution and 300 µl Buffer GHL, shake and mix, and lyse at 75°C for 15 min。During this time, invert and mix 3 times, 3-5 times each time.

3. Follow the part 2.

B. Saliva

1. Take 300 µl of saliva sample into a 1.5 ml centrifuge tube, add 300 µl of Buffer GHL and 20 µl of Proteinase K, shake and mix, lyse at 75°C for 15 min or 65°C for 20 min, invert and mix 3 times during each time. Go back 3-5 times.

2. Follow the part 2.

C. Swab

1. Sample processing:

1) Dry swab sample: After sample collection, add 500 µl of tissue digestion solution MDA， vortex for 10 sec to mix, take 300 µl sample into a 1.5 ml centrifuge tube, add 300 µl Buffer GHL 和 20 µl Proteinase K.

2) Swab sample with preservation solution: Take 300 µl of the swab sample into a 1.5 ml centrifuge tube, add 300 µl Buffer GHL and 20 µl Proteinase K, and vortex for 10 sec to mix.

2. Lyse at 75°C for 15 minutes, inverted and mixed for 3 times, 3-5 times each time.

3. Follow the part 2.

D. Tissue

1. Take 10-20 mg of animal tissue, cut into small pieces as much as possible, add 300 µl Buffer MDA and 20 µl Proteinase K, and use an electric homogenizer to grind the tissue until the tissue is fully grinded.

1) For samples with sufficient homogenization, the digestion time can be omitted.

2) For samples with visible tissue clumps or rat tail samples , it is recommended to digest at 75°C for 30 min or more time, until the digestion is complete.

Note: After the sample is digested, if there are tissue fragments, it is recommended to centrifuge at 12,000 rpm for 1 min to remove residual impurities. If you need to remove RNA, add 4µl RNase A and leave it at room temperature for 10 min (GENFINE, B027, Self-prepared).

2. After digestion, add 300 µl Buffer GHL to the sample, shake and mix.

3. Follow the part 2.

E. Bacteria

1. Take 1-2 ml of bacterial culture solution, centrifuge at 10,000 rpm (~11,500×g) for 1 min, and discard the supernatant.

2. Add 300 µl Buffer MDA to the bacterial pellet, and shake until the bacterial cells are completely suspended.

Note: For gram-positive bacteria that are difficult to break, skip the second step and add lysozyme (20mg/ml) to break the wall and treat it at 37 ℃ for more than 30 minutes. If RNA needs to be removed, 4 µl RNase A (100 mg/ml) solution (GENFINE, B027, self-prepared) can be added to the suspension in the previous step and shake for 30 seconds, and leave it at room temperature for 10 minutes.

3. Add 300 µl Buffer GHL and 20 µl Proteinase K to the suspension in the previous step, shake until the bacteria are completely suspended, lyse at 75°C for 15-20 minutes or at 65°C for 20-30 minutes, if the bacteria are not clear, it can be extended appropriately The lysis time is until the bacteria becomes clear. During this period, mix upside down for 3-5 times, each time 3-5 times.

4. Follow the part 2.

F. Cell sample

1. Use 300 µl Buffer MDA and 20 µl Proteinase K to suspend and shake the cell pellet with a cell number of about 1×10^6-10^7.

2. Add 300 µl Buffer GHL, shake until the cells are completely suspended, lyse at 75°C for 15 minutes until it becomes clear. During this period, mix upside down for 3-5 times, 3-5 times each time.

3. Follow the part 2.

Part 2：DNA purification

I. Manual Operation

1. Take out 200-500 µl of the digested sample to a new 1.5 ml centrifuge tube, directly add 300 µl Buffer IP and 25 µl FineMag Particles GF. Shake and mix for 1 min, let stand for 9 min in total, shake and mix for 1 min every 3 min.

Note: To ensure that the magnetic beads are completely resuspended, shake and mix before use.

2. Place the centrifuge tube on the Magnetic Separation Rack for 30 sec until all the magnetic particles are cleared from the solution. Discard the supernatant carefully.

3. Add 900 µl Buffer MW and mix by vortex for 2 min.

4. Place the centrifuge tube on the Magnetic Separation Rack for 30 sec until all the magnetic particles are cleared from the solution. Discard the supernatant carefully.

5. Add 500 µl Buffer MW and mix by vortex for 2 min.

6. Place the centrifuge tube on Magnetic Separation Rack for 30 sec until all the magnetic particles are cleared from the solution. Discard the supernatant carefully.

7. Remove the centrifuge tube from the Magnetic Separation Rack, add 900 µl Buffer MWP, mix by vortex for 1 min.

8. Place the centrifuge tube on the Magnetic Separation Rack for 30 sec until all the magnetic particles are cleared from the solution. Discard the supernatant carefully.

9. Remove the centrifuge tube from the Magnetic Separation Rack, add 300 µl Buffer MWP, mix by vortex for 1 min.

10. Place the centrifuge tube on the Magnetic Separation Rack for 30 sec until all the magnetic particles are cleared from the solution. Discard the supernatant carefully.

11. Place the centrifuge tube on the Magnetic Separation Rack and air dry at the room temperature for 10-15 min.

Note: Residual ethanol may inhibit subsequent enzymatic reactions. Please ensure the residual ethanol is removed completely. However, overdrying should be avoided, since over-dried DNA is difficult to dissolve.

12. Remove the centrifuge tube from the Magnetic Separation Rack, add 50-100 µl Buffer EB, shake and mix, heat at 56℃ for 10 min and shake and mix for 2 min every 3-5 min of heating.

13. Place the Centrifuge tube on the Magnetic Separation Rack for 2 min until all the magnetic particles are cleared from the solution. Transfer the supernatant containing DNA to a new centrifuge tube and store. It can be stored at 4°C temporarily if it is tested immediately, and stored at -80°C for long-term use.

II. Automated purification (for GENFINE Purifier 32)

1. Take out pre-filled 96-well plates from the box, gently upside down to mix the beads. Carefully remove the seals from pre-filled plates to prevent liquid from spilling.

2. Take 200-500 µl of the processed sample of the first part, add sample to rows 2/8 of a 96-well plate(**If it is a single reagent, the arc position starts to be recorded as the row 1**).

3. Place the 96-well plates and 8-rod combs into the right position of the GENFINE Purifier 32 .

3. Select the program“Y202-G10”and start to run on the instrument.

4. At the end of the run, immediately remove 96-well plates from the instrument, then transfer the solution of rows 6, 12 to the final tube and store.

Table 2. Protocol of “Y202-G10” for Purifier 32

Step	Well	Name	Mix time (s)	Amplitude	Frequency	Magnetic time (s)	Cycles	Stay time (s)	Volume (μl)	Temperature (℃)	Heat time (s)
1	1	Binding	30	中	快	30	1	0	400	--	0
2	2	Binding	600	中	中	30	2	0	800	--	0
3	3	Washing	180	中	中	30	1	0	800	--	0
4	4	Washing	180	中	中	30	1	0	800	--	0
5	5	Washing	180	中	中	30	1	300	800	--	0
6	6	Elution	600	高	快	30	4	0	100	75	600
7	1	Discard Beads	30	中	快	--	--	0	400	--	0

III. Automated purification (for GENFINE P96)

1. Take out prefilled 96-well plates from the box, gently upside down to mix the beads. Flick downward or gently tap each plate before removing the seal.

2. Take 200-500 μl of the processed sample of the first part, add sample to Plate 2 (Buffer IP).

Note: The sample needs to be equilibrated to room temperature and run the program within 1h after adding the sample.

3. Put the 96 Tip Comb into the Plate 1 (Buffer DP+MGF).

4. Immediately load the remaining plates onto the instrument as prompted.

5. Select the program"Y202-G30"and start the run.

6. At the end of the run, immediately remove the Plate 6 (Buffer EB) from the instrument, then transfer the solution to the final tubes/plate and store.

Note: The purified nucleic acid is ready for immediate use. Alternatively, store the plate at –20°C for long term storage.

Table 3. Protocol of "Y202-G30" for P96

Step	Well	Name	Volume (μl)	Temperature		Stay		Position	Shock		Magnetic			
				Value (℃)	ON/OFF	Mode	Time (s)		Time (s)	Strength	Position	Time (s)	Cycle	Mode
1	1	Load	--	--	--	--	--	--	--	--	--	--	--	--
2	1	Binding	400	--	--	--	--	90%	30	High	1	20	2	Step-by-step
3	2	Binding	900	56	ON	--	--	90%	600	High	1	30	3	Step-by-step
4	3	Washing	800	--	--	--	--	90%	180	High	1	20	2	Step-by-step
5	4	Washing	800	--	--	--	--	90%	180	High	1	20	2	Step-by-step
6	5	Washing	800	--	--	--	--	90%	180	High	1	20	2	Step-by-step
7	5	Elution	800	75	ON	Stay	300	--	--	--	--	--	--	--
8	6	Elution	100	75	ON	/	/	90%	600	High	1	30	4	Step-by-step
9	1	Discard Beads	--	--	--	--	--	--	--	--	--	--	--	--

[Precautions]

1. Please read the instructions carefully before use and operate in strict accordance with the requirements.
2. The operators can take up the post only after relevant trainings.
3. Note not to cause cross contamination during sample operation.
4. It is normal if there is crystallization in the lysate. Please incubate the lysate at 50~60°C for 30 minutes until the crystallization is completely melted, shake well, and cool to room temperature before use.

5. Failure to follow the instructions will result in inaccurate results

6. Please do not use the product beyond the expiration date, and do not mix the reagent components of different batch numbers.

7. The samples to be tested involved in the kit should be considered as infectious substances, and should be handled and treated according to the requirements of *General Rules for Safety of Microorganism and Biomedical Laboratory* and *Regulations for Management of Medical Wastes*.

Symbols	Meanings
	Manufacturer
	Authorized representative in the European Community
	This product fulfills the requirements of the European Directive 98/79 EC for in vitro diagnostic medical devices.
	Catalogue number
	Batch code
	Date of manufacture
	Use-by date
	Temperature limit
	Consult instructions for use
	Keep dry
	Keep away from sunlight
	Do not re-use
	Do not use if package is damaged

[Symbols]

GENFINE BIOTECH (CHANGZHOU) CO., LTD.
4th Floor, Building E4, No.9, Changyang Road, West Taihu Technology Industrial Park, Changzhou City, Jiangsu Province
PEOPLE'S REPUBLIC OF CHINA
Tel: +86 051983761557
E-mail: marketing@genfine.com
Web: en.genfine.com

Lotus NL B.V.
Koningin Julianaplein 10, 1e Verd, 2595AA, The Hague, Netherlands.
E-mail：peter@lotusnl.com
Tel： +31644168999