HB3700

Ribospin™ Plant

PLANT TOTAL RNA PURIFICATION HANDBOOK

GeneAll

Customer & Technical Support

Should you have any further questions, do not hesitate to contact us.

We appreciate your comments and advice.

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This protocol handbook is included in :

GeneAll® Ribospin™ Plant (307-150)

Visit www.geneall.com or www.geneall.co.kr for FAQ, Q&A and more information.

Brief Protocol

Homogenization

1. Prepare 100 mg plant samples

Lyse

2. Add 350 µl Buffer RPL
3. Incubate for 3 min at RT

EzPure™ Filter

4. Transfer the lysate into EzPure™ Filter
5. Centrifuge for 30 sec, ≥10,000 x g
6. Transfer the supernatant into a new microcentrifuge tube

Bind

7. Add 1 vol. (µl) 70% EtOH to 1 vol. (µl) supernatant
8. Apply the mixture into mini column
9. Centrifuge for 30 sec, ≥10,000 x g

DNase I treatment

10. Add 500 µl Buffer RBW into mini column
11. Centrifuge for 30 sec, ≥10,000 x g
12. Apply 70 µl DNase I reaction mixture into mini column
13. Incubate for 10 min at RT

Wash

14. Add 500 µl Buffer RBW into mini column
15. Incubate for 2 min at RT
16. Centrifuge for 30 sec, ≥10,000 x g
17. Add 500 µl Buffer RNW into mini column
18. Centrifuge for 30 sec, ≥10,000 x g
19. Repeat step 17~18
20. Additional centrifuge for 1 min, ≥10,000 x g

Elute

21. Apply 50 µl Nuclease-free water into mini column
22. Incubate for 1 min at RT
23. Centrifuge for 1 min, ≥10,000 x g

Pure DNA

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KIT CONTENTS

Cat. No.	307-150	Storage
Components	Quantity	
No. of preparation	50	Room temperature (15~25°C)
Buffer RPL	25 ml	
Buffer REL	25 ml	
Buffer RBW (concentrate) *	27 ml	
Buffer RNW (concentrate) * †	12 ml	
Nuclease-free water	15 ml	
Buffer DRB	5 ml	
EzPure™ Filter (mini) (with collection tube)	50	
Column Type W (mini) (with collection tube)	50	
1.5 ml microcentrifuge tube	100	
Protocol Handbook	1	
DNase I ** **(lyophilized)**	240 Kunitz units	-20°C

** Before first use, add absolute ethanol (ACS grade or better) into Buffer RBW and RNW as indicated on the bottle.*

† Contains sodium azide as a preservative.

*** Refer to instruction of DNase I on page 10.*

Materials Not Provided

Reagent : 70% ethanol, Absolute ethanol (ACS grade or better)
Disposable material : RNase-free pipette tips, Disposable gloves
Equipment : Microcentrifuge, Vortex mixer, Equipment for disrupting plant tissue

Product Specifications

Ribospin™ Plant	
Type	Spin
Maximum amount of starting samples	100 mg/prep
Preparation time	≥30 min
Maximum loading volume of mini column	750 μl
Minimum elution volume	30 μl
Maximum binding capacity	~100 μg

Quality Control

All components in Ribospin™ Plant are manufactured in strictly clean conditions, and its degree of cleanness is monitored periodically. Quality control is carried out thoroughly from lot to lot, and only the qualified kits are approved to be delivered.

Storage Conditions

All components of Ribospin™ Plant should be stored at room temperature (15~25°C). It should be protected from exposure to direct sunlight. DNase I should be stored at -20°C after reconstitution. During shipment or storage under cool ambient condition, a precipitate can be formed in Buffer RPL, REL, RBW. In such a case, heat the bottle to 50°C to dissolve completely. Using precipitated buffers will lead to poor RNA recovery. Ribospin™ Plant is guaranteed until the expiration date printed on the product box.

Safety Information

The buffers included in Ribospin™ Plant kit contain irritant which is harmful when in contact with skin or eyes, or when inhaled or swallowed. Care should be taken when handling such materials. Always wear gloves and eye protector, and follow standard safety precautions. Buffer RPL, REL, and RBW contain chaotropic agents, which can form highly reactive compounds when combined with bleach. Do NOT add bleach or acidic solutions directly to the sample-preparation waste.

Product Disclaimer

Ribospin™ Plant is for research use only, not for use in diagnostic procedure.

Preventing RNase Contamination

RNase can be introduced accidentally during RNA purification. Wear disposable gloves always, because skin often contains bacteria and molds that can be a source of RNase contamination. Use sterile, disposable plastic wares and automatic pipettes to prevent cross-contamination of RNase from shared equipment.

Typical Yields

	Sample type	Amount of starting material	Typical yield
Leaf	*Pinus densiflora* (Pine)	100 mg	2.7 μg
	Cucumis sativus L. (Cucumber)	100 mg	50 μg
	Zea mays (Corn)	100 mg	11 μg
	Capsicum annuum (Red pepper)	100 mg	22 μg
	Lycopersicum esculentum (Tomato)	50 mg	13 μg
	Lactuca sativa (Lettuce)	100 mg	29 μg
	Citrus grandis Osbek (Satsuma)	100 mg	4.6 μg
	Diospyros kaki (Persimmon)	100 mg	16 μg
	Crassula ovata (Crassula)	100 mg	3 μg
	Nicotiana tabacum (Tobacco)	50 mg	13 μg
Root	*Allium cepa* (Onion)	100 mg	8 μg
	Plantago asiatica (Plantain)	50 mg	2.5 μg
	Nicotiana tabacum (Tobacco)	50 mg	5.3 μg
Fruit	*Citrus grandis Osbek* (Satsuma)	50 mg	1.1 μg
Germ bud	*Allium cepa* (Onion)	100 mg	9 μg

Product Description

Ribospin™ Plant kit is specially designed for purification of total RNA from various plant tissues such as leaves, stems, roots and picky plant samples. This kit provides the optimized buffer and spin column, which is effective at removing polysaccharides and polyphenolic compounds and isolating intact plant RNA. All components of Ribospin™ Plant are ready for use, so any further preparation for experiment is not required.

The procedure of Ribospin™ Plant kit begins with the disruption of sample in liquid nitrogen using mortar and pestle. The disrupted sample can be lysed in Buffer RPL or REL. In most case, Buffer RPL is the best buffer for lysis. However in some plant samples, solidification of lysate can be occurred with Buffer RPL due to endosperm of seed or peculiar metabolites, and this can be avoided by using Buffer REL as alternative for Buffer RPL.

Most impurities except RNA in the lysate are eliminated by filtration through EzPure™ Filter, and then the passed-through lysate is mixed with ethanol to adjust binding condition. Total RNA including a little impurity is bound to the membrane of Column Type W while the mixture is passing through. Survived genomic DNA can be exterminated by on-column DNase I treatment at this step. After a series of washing step using Buffer RBW and RNW, plant total RNA is eluted by Nuclease-free water.

Whole procedure of Ribospin™ Plant takes only 25 minutes. The purified RNA is suitable for cDNA synthesis, RT-PCR, Northern blotting, and other analytical procedure.

PROTOCOL FOR

Ribospin™ Plant

Before experiment

- Before using for the first time, add absolute ethanol (ACS grade or better) into Buffer RBW and RNW as indicated on the bottle.

- Prepare DNase I reaction mixture just before step 12.

v Prepare aliquot DNase I and thaw on ice.

v Mix 2 µl DNase I with 70 µl Buffer DRB.

1. **Prepare plant tissue sample up to 100 mg, then grind the sample to a fine powder using a mortar and pestle with liquid nitrogen and transfer the grinded sample into a 1.5 ml microcentrifuge tube (not provided).**

2. **Add 350 µl of Buffer RPL to the 1.5 ml microcentrifuge tube and vortex vigorously.**
   In case of solidification of the lysate in Buffer RPL, use Buffer REL instead of Buffer RPL.

3. **Incubate 3 min at room temperature.**

4. **Transfer the lysate to a EzPure™ Filter.**
   Through this step, large cell debris and most of genomic DNAs are filtered on the EzPure™ Filter and small pellet of cell debris will be formed at the bottom of the collection tube.

5. **Centrifuge at ≥10,000 x g for 30 sec at room temperature.**

6. **Transfer the supernatant to a new 1.5 ml microcentrifuge tube (provided).**
   Be careful not to disturb the pellet at the bottom of the collection tube.

7. **Add 1 volume (usually 350 µl) of 70% EtOH to the tube containing supernatant, and mix well by pipetting or inverting.**
   Do not centrifuge at this step.

8. **Apply the mixture to a Column Type W (blue ring).**

9. **Centrifuge at ≥10,000 x g for 30 sec at room temperature. Discard the pass-through and reinsert the mini column back into the collection tube.**

10. **Add 500 μl of Buffer RBW to the mini column.**

11. **Centrifuge at ≥10,000 x g for 30 sec at room temperature. Discard the pass-through and reinsert the mini column back into the collection tube.**

12. **Apply 70 μl of DNase I reaction mixture to the center of the mini column. Incubate at the room temperature for 10 minutes.**
    To make DNase I reaction mixture, mix 2 μl DNase I solution with 70 μl Buffer DRB per isolation. DNase I is sensitive to physical damage. Therefore, do not mix vigorously. If you want to DNase I treatment in RNA eluate, skip step 12 and 13 and refer to Appendix I "DNase I treatment in eluate".

13. **Add 500 μl of Buffer RBW to the mini column and stand for 2 min.**
    Buffer RBW inactivates DNase I and wash out the components of DNase I reaction buffer.

14. **Centrifuge at ≥10,000 x g for 30 sec at room temperature. Discard the pass-through and reinsert the mini column back into the collection tube.**

15. **Add 500 μl of Buffer RNW to the mini column.**

16. **Centrifuge at ≥10,000 x g for 30 sec at room temperature. Discard the pass-through and reinsert the mini column back into the collection tube.**

17. **Repeat step 15~16.**

18. **Centrifuge at ≥10,000 x g for an additional 1 min at room temperature to remove residual wash buffer. Transfer the mini column to a new 1.5 ml microcentrifuge tube (provided).**
    Residual ethanol may interfere with downstream reactions. Care must be taken at this step for eliminating the carryover of Buffer RNW.

19. Add 50 μl of Nuclease-free water to the center of the membrane in the mini column.

To increase the RNA concentration, reduce the elution volume to 30 μl.

20. Centrifuge at ≥10,000 x g for 1 min at room temperature.

Purified RNA can be stored at 4°C for immediate analysis and can be stored at -70°C for long term storage.

The purified RNA is free of DNA and proteins, and A_{260}/A_{280} will be between 1.8 and 2.2.

Troubleshooting Guide

Facts	Possible Causes	Suggestions
Low yield of RNA	**Insufficient Grinding of the sample**	Insufficient disruption can lead to decrease the yield of total RNA. Confirm the completley disrupted sample in liquid nitrogen and transfer the disrupted sample in a 1.5 ml microcentrifuge tube.
	Too much starting sample	Overloading can decrease the yield of total RNA. Reduce the amount of starting sample.
	Poor quality of starting material	Process the sample immediately after harvest. To process later, freeze the sample rapidly in liquid nitrogen.
	Too low RNA mass in samples	Especially, some plant samples have low RNA content. To increase the RNA concentration, reduce the elution volume up to 30 μl or increase the amount of starting sample up to 100 mg per prep.
RNA degradation	**Too much manipulated sample before process**	Process the sample immediately after harvest. To process later, freeze the sample rapidly in liquid nitrogen.
	Improper storage of extracted RNA	Store isolated RNA at -70°C, Do not store at -20°C.
	Reagent or disposable is not RNase-free	Make sure to use RNase-free products only.
EzPure™ Filter clogging	**Insufficient Grinding of the sample**	Insufficient disruption can clog the EzPure™ Filter and to decrease the yield of total RNA. Confirm the complete disruption of the sample in liquid nitrogen.

Troubleshooting Guide

Facts	Possible Causes	Suggestions
DNA contamination of RNA eluate	**Too much starting sample**	Too much starting sample may leave lots of DNA fragments on the membrane over the activity of DNase I. Reduce starting sample used.
	High DNA mass in sample	Some plant samples have high DNA content. In this case, some DNA can be eluted at RNA elution step. Reducing the amount of sample can reduce the genomic DNA contamination or refer to the appendix I 'DNase I treatment in eluate'.
	Inactivation of DNase I	For prolonged activity, aliquot the DNase I into small portion. Do not freezing and thawing the aliquots several times.
	Incorrect DNase I reaction treatment	Add DNase I reaction mixture to the center of the mini column membrane.
Eluate does not perform well in downstream application	**Residual ethanol remains in eluate**	To remove any residual ethanol included in Buffer RNW from mini column membrane, centrifuge again for complete removal of ethanol.
	Buffer RBW and RNW used in wrong order	Ensure that Buffer RBW and RNW are used in correct order. If used in the wrong order, wash the mini column with Buffer RNW finally.

APPENDIX I. DNase I treatment in eluate

Appendix I describe how to use the DNase I (included in this kit) to eliminate contaminating DNA in RNA eluate. For samples containing high DNA contents, this method is strongly recommended. This procedure is more efficient than on-column DNase I treatment.

Protocol

1. Prepare the mixture as below in a 1.5 ml microcentrifuge tube.
50 μl RNA eluate
5 μl Buffer DRB
1 μl DNase I

2. Incubate the mixture for 10 min at room temperature.

3. Re-elution of RNA.
Follow 3-1 or 3-2

DNase I treated RNA can be applied to RNA clean up kit (Riboclear™ Cat. No. 303-150). We strongly recommend using Riboclear™ kit for RNA clean up. Because ethanol precipitation and heat inactivation, usually used for DNase I inactivation, can damage the RNA.

1 Follow Riboclear™ protocol

2 Heat inactivation
1. Add 1 μl of 0.5 M EDTA per 100 μl eluate.
2. Heat inactivate at 75°C for 10 min.

APPENDIX II. Confirmation of RNA yield and purity by UV absorbance

Concentration of RNA

The concentration of RNA can be determined by the absorbance at 260 nm using spectrophotometer. For the convenient measurement, we recommend using the NanoDrop® which can reduce your RNA sample and time. If not, you need to dilute the RNA samples to measure the concentration through traditional spectrophotometer. The value of A_{260} should be between 0.15 and 1.00. Be sure to calibrate the spectrophotometer with the same solution used for dilution. An absorbance of 1 at 260 nm is correspond to about 40 μg RNA/ml at a neutral pH. Therefore, the concentration of RNA was calculated by the formula shown below.

A_{260} x dilution factor x 40 = RNA μg/ml

Purity of RNA

To confirm the RNA purity, you should read the ratio of A_{260}/A_{280}. Pure RNA is in the range of 1.8~2.2.

APPENDIX III. Formaldehyde agarose gel electrophoresis (Denaturing gel method)

A denaturing agarose gel is routinely used for the assessment of the quality of an RNA preparation. After preparation, RNA forms secondary structure via intramolecular base pairing. Therefore, it is very difficult to get the exact result of electrophoresis because of migrating inaccuracy. However, the denaturing gel denatures the secondary structure of RNA and makes an accurate migration.

To confirm the RNA band, the gel should be transferred to a UV transilluminator after electrophoresis. Mainly, two RNA bands are shown. In case of animal sample, the 28S and 18S rRNA bands are confirmed on the gel. If they are intact, the RNA bands should be sharp and the intensity of upper band should be about twice that of the lower band.

Prepare the denaturing gel

1. Put 1 g agarose in 72 ml water and heat to dissolve thoroughly.
2. Cool to 60°C.
3. Add 10 ml of 10X MOPS buffer, 18 ml of 37% formaldehyde, and 1 μl of a 10 mg/ml ethidium bromide (EtBr).
4. Mix well then pour the gel into the gel tray and cool to solidify it.
5. Transfer the solidified gel from tray to tank, and add enough 1X MOPS running buffer to cover the gel.

Prepare the RNA sample

1. Make the mixture.
   x μl RNA (up to 20 μg)
   2 μl 10X MOPS electrophoresis buffer
   4 μl Formaldehyde
   10 μl Formamide
2. Incubate the mixture for 15 min at 65°C.
3. Chill the sample for 5 min on ice.
4. Add 2 μl of 10X formaldehyde gel-loading dye to the mixture.
5. Load the mixture in a denaturing gel which is covered with a sufficient 1X MOPS electrophoresis buffer.
6. Run the gel and confirm the RNA band on transilluminator.
   Occasionally, gel destaining may be needed to increase the visibility of the bands of RNA in dH_2O for several hours.

Composition of buffers

- 10X MOPS buffer
  0.2 M MOPS
  20 mM Sodium acetate
  10 mM EDTA
  pH to 7.0 with NaOH

- 10X formaldehyde gel-loading dye
  50% Glycerol
  10 mM EDTA
  0.25% (w/v) Bromophenol blue
  0.25% (w/v) Xylene cyanol FF

*** Caution**

When working with these chemicals, always use gloves and eye protector to avoid contact with skin and cloth. Especially, formaldehyde and ethidium bromide (EtBr) should be handled in a fume hood.

Ordering Information

GeneAll® *Hybrid-Q™* *for rapid preparation of plasmid DNA*

Products	Scale	Size	Cat. No.	Type
Plasmid Rapidprep	mini	50	100-150	spin
		200	100-102	

GeneAll® *Exprep™* *for preparation of plasmid DNA*

Products	Scale	Size	Cat. No.	Type
Plasmid SV	mini	50	101-150	spin / vacuum
		200	101-102	
	Midi	26	101-226	spin / vacuum
		50	101-250	
		100	101-201	

GeneAll® *Exfection™*
for preparation of transfection-grade plasmid DNA

Products	Scale	Size	Cat. No.	Type
Plasmid LE *(Low Endotoxin)*	mini	50	111-150	spin / vacuum
		200	111-102	
	Midi	26	111-226	spin / vacuum
		100	111-201	
Plasmid EF *(Endotoxin Free)*	Midi	20	121-220	spin
		100	121-201	

GeneAll® *Expin™* *for purification of fragment DNA*

Products	Scale	Size	Cat. No.	Type
Gel SV	mini	50	102-150	spin / vacuum
		200	102-102	
PCR SV	mini	50	103-150	spin / vacuum
		200	103-102	
CleanUp SV	mini	50	113-150	spin / vacuum
		200	113-102	
Combo GP	mini	50	112-150	spin / vacuum
		200	112-102	

GeneAll® *Exgene™* *for isolation of total DNA*

Products	Scale	Size	Cat. No.	Type
Tissue SV	mini	100	104-101	spin / vacuum
		250	104-152	
	Midi	26	104-226	spin / vacuum
		100	104-201	
	MAXI	10	104-310	spin / vacuum
		26	104-326	
Tissue *plus!* SV	mini	100	109-101	spin / vacuum
		250	109-152	
	Midi	26	109-226	spin / vacuum
		100	109-201	
	MAXI	10	109-310	spin / vacuum
		26	109-326	

GeneAll® *Exgene™* *for isolation of total DNA*

Products	Scale	Size	Cat. No.	Type
Blood SV	mini	100	105-101	spin / vacuum
		250	105-152	
	Midi	26	105-226	spin / vacuum
		100	105-201	
	MAXI	10	105-310	spin / vacuum
		26	105-326	
Cell SV	mini	100	106-101	spin / vacuum
		250	106-152	
	MAXI	10	106-310	spin / vacuum
		26	106-326	
Clinic SV	mini	100	108-101	spin / vacuum
		250	108-152	
	Midi	26	108-226	spin / vacuum
		100	108-201	
	MAXI	10	108-310	spin / vacuum
		26	108-326	
Genomic DNA micro		50	118-050	spin
Plant SV	mini	100	117-101	spin / vacuum
		250	117-152	
	Midi	26	117-226	spin / vacuum
		100	117-201	
	MAXI	10	117-310	spin / vacuum
		26	117-326	
Soil DNA mini	mini	50	114-150	spin
Stool DNA mini	mini	50	115-150	spin
Viral DNA / RNA	mini	50	128-150	spin
FFPE Tissue DNA	mini	50	138-150	spin
		250	138-152	

GeneAll® *GenEx™* *for isolation of total DNA without spin column*

Products	Scale	Size	Cat. No.	Type
GenEx™ Blood	Sx	100	220-101	solution
		500	220-105	
	Lx	100	220-301	solution
GenEx™ Cell	Sx	100	221-101	solution
		500	221-105	
	Lx	100	221-301	solution
GenEx™ Tissue	Sx	100	222-101	solution
		500	222-105	
	Lx	100	222-301	solution

Products	Scale	Size	Cat. No.	Type

GeneAll® *GenEx™* *for isolation of total DNA*

GenEx™ Plant	Sx	100	227-101	solution
	Mx	100	227-201	
	Lx	100	227-301	
GenEx™ Plant *plus!*	Sx	100	228-101	solution
	Mx	50	228-250	
	Lx	20	228-320	

GeneAll® *DirEx™* series
for preperation of PCR-template without extraction

DirEx™	100	250-101	solution
DirEx™ *Fast*-Tissue	96 T	260-011	solution
DirEx™ *Fast*-Cultured cell	96 T	260-021	solution
DirEx™ *Fast*-Whole blood	96 T	260-031	solution
DirEx™ *Fast*-Blood stain	96 T	260-041	solution
DirEx™ *Fast*-Hair	96 T	260-051	solution
DirEx™ *Fast*-Buccal swab	96 T	260-061	solution
DirEx™ *Fast*-Cigarette	96 T	260-071	solution

GeneAll® RNA series *for preperation of total RNA*

RiboEx™	mini	100	301-001	solution
		200	301-002	
Hybrid-R™	mini	100	305-101	spin
Hybrid-R™ Blood RNA	mini	50	315-150	spin
Hybrid-R™ miRNA	mini	50	325-150	spin
RiboEx™ LS	mini	100	302-001	solution
		200	302-002	
Riboclear™	mini	50	303-150	spin
Riboclear™ *plus!*	mini	50	313-150	spin
Ribospin™	mini	50	304-150	spin
Ribospin™ II	mini	50	314-150	spin
		300	314-103	
Ribospin ™ vRD	mini	50	302-150	spin
Ribospin ™ vRD *plus!*	mini	50	312-150	spin
Ribospin ™ vRD II	mini	50	322-150	spin
Ribospin ™ Plant	mini	50	307-150	spin
Ribospin™ Seed / Fruit	mini	50	317-150	spin
Allspin™	mini	50	306-150	spin
RiboSaver™	mini	100	351- 001	solution

Products	Scale	Size	Cat. No.	Type

GeneAll® *AmpONE™* *for PCR amplification*

Taq DNA polymerase		250 U	501-025	(2.5 U/μℓ)
		500 U	501-050	
		1,000 U	501-100	
Taq Premix	96 tubes	20 μℓ	526-200	solution
		50 μℓ	526-500	

GeneAll® *AmpMaster™* *for PCR amplification*

Taq Master mix	0.5 ml x 2 tubes	541-010	solution
	0.5 ml x 10 tubes	541-050	solution

GeneAll® *HyperScript™* *for Reverse Transcription*

Reverse Transcriptase	10,000 U	601-100	solution
RT Master mix	0.5 ml × 2 tubes	601-710	solution
One-step RT-PCR Master mix	0.5 ml × 2 tubes	602-110	solution
One-step RT-PCR Premix	96 tubes, 20 μℓ	602-102	solution

GeneAll® RealAmp™ *for qPCR amplification*

SYBR qPCR Master mix (2X, Low ROX)	200 rxn	20 μℓ	801-020	solution
	500 rxn	20 μℓ	801-050	
SYBR qPCR Master mix (2X, High ROX)	200 rxn	20 μℓ	801-021	solution
	500 rxn	20 μℓ	801-051	

Products	Size	Cat. No.	Type
GeneAll® Protein series			
ProtinEx™ Animal cell / tissue	100 ml	701-001	solution
PAGESTA™ Reducing 5X SDS-PAGE Sample Buffer	1 ml × 10 tubes	751-001	solution
GeneAll® STEADi™ *for automatic nucleic acid purification*			
12 Instrument		GST012	system
24 Instrument		GST024	system
Genomic DNA Cell / Tissue	96	401-104	kit
Genomic DNA Blood	96	402-105	kit
Total RNA	96	404-304	kit
Viral DNA / RNA	96	405-322	kit
CFC Seed DNA / RNA	96	406-C02	kit
Genomic DNA Plant	96	407-117	kit
Soil DNA	96	408-114	kit
GeneAll® GENTi™ 32 *Ultimately flexible automatic extraction system*			
Automatic extrantion equipment		GTI032	system
Genomic DNA	48	901-048	strip
	96	901-096	plate
Viral DNA / RNA	48	902-048	strip
	96	902-096	plate
Whole Blood Genomic DNA	48	903-048	strip
	96	903-096	plate

Note

Note

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