

FavorPrep™ Tissue Total RNA Extraction Mini Kit

For Research Use Only

-For isolation RNA from animal cells, animal tissues, bacteria, yeast, paraffin fixed sample, fungi and for RNA clean up.

Kit Contents:

Cat. No:	FATR1020 (4 preps)	FATR1023 (50 preps)
FARB Buffer	1.5 ml × 2	25 ml
Wash Buffer 1	1.5 ml × 2	30 ml
Wash Buffer 2 (Concentrate)	1.5 ml	20 ml
RNase-Free Water	0.5 ml	6 ml
Filter Columns	4 pcs	50 pcs
FARB Mini Columns	4 pcs	50 pcs
Collection Tubes	8 pcs	100 pcs
Elution Tubes	4 pcs	50 pcs
Micropestles	4 pcs	50 pcs
User Manual	1	1

■ Adding Ethanol to the concentrate Wash Buffer 2.
See Working Buffer Preparation.

Cat. No:	FATR1024 (100 preps)	FATR1026 (300 preps)
FARB Buffer	45 ml	130 ml
Wash Buffer 1	60 ml	170 ml
Wash Buffer 2 (concentrate)	35 ml	50 ml × 2
RNase-Free Water	6 ml	8 ml × 2
Filter Columns	100 pcs	300 pcs
FARB Mini Columns	100 pcs	300 pcs
Collection Tubes	200 pcs	600 pcs
Elution Tubes	100 pcs	300 pcs
Micropestles	100 pcs	300 pcs
User Manual	1	1

■ Adding Ethanol to the concentrate Wash Buffer 2.
see Working Buffer Preparation.

Sample amount and yield:

Sample	Recommended amount of sample used	Yield (µg)
Animal cells (up to 5×10 ⁶)	NIH/3T3	10
	HeLa	15
	COS-7	30
	LMH	12
Animal Tissue (Mouse/rat) (up to 30 mg)	Embryo	25
	Heart	10
	Brain	10
	Kidney	30
	Liver	50
	Spleen	35
	Lung	15
Thymus	45	
Bacteria	E. coli	60
	B. subtilis	40
Yeast (up to 5×10 ⁷)	S. cerevisiae	25

Important Note:

Notes for Buffers:

- Make sure that the working environment is RNase-free.
- Buffers provided by this kit containing irritants, wear gloves and lab coat for operation.
- Caution: β-mercaptoethanol (β-Me) is hazardous to human health. perform the procedures involving β-Me in a chemical fume hood.**
- The centrifuge force should be performed at ~18,000 xg.
- The vacuum source should be reached to -6 inches Hg.
- Add ethanol (96~100%) to concentrate Wash Buffer 2 before use. see **Working Buffer Preparation.**
- For handling the buffers safely please read **safety Information** before starting the procedure.

2. Notes for centrifuging and vacuum:

- When using of vacuum to operate the RNA extraction, ensure that the tip of the column is fit into the shape of manifold adaptor, and the vacuum pressure being capable to reach to -6 inches Hg.
- Units and values at same pressure (1 atm)

unit	value
atmosphere (atm)	1.000
millimeter of mercury (mmHg)	760.000
inches of mercury (inHg)	29.290
pascal (Pa)	101,325.000
kilopascal (kPa)	101.325
torr (torr)	760.000
pound per square inch (psi, lbs/in ²)	14.700

Materials and equipment provided by the user

For All Protocol:

- Pipets, pipet tips and centrifuge tubes (1.5 ml, 2.0 ml), sterile
- RNase-free 96~100% ethanol (for preparation of Wash Buffer 2).

For centrifuge processing:

- A micro-centrifugator is capable of ~18,000 xg, with a rotor for 1.5 or 2.0 ml micro-centrifuge tube.

For vacuum processing:

- A micro-centrifugator is capable of ~18,000 xg, with a rotor for 1.5~2.0 ml micro-centrifuge tube.
- A vacuum manifold contains adaptors for Filter Columns and FARB Column. The vacuum be capable to -6 inches Hg.

Working Buffer Preparations:

1. "■" Preparation of Wash Buffer 2

Add RNase-free ethanol (96~100%) to the concentrate Wash Buffer 2 as the table indicated. Store the ethanol-added Wash Buffer 2 at 15~25°C.

Cat. No./ preps	Ethanol volume to Wash Buffer 2
FATR1020/ 4 preps	6 ml
FATR1023/ 50 preps	80 ml
FATR1024/ 100 preps	140 ml
FATR1026/ 300 preps	200 ml

2. Preparation of "RNase-free" DNase I reaction solution for Optional Step, On-Column DNase I Digestion.

For each reaction, prepare 60 µl of RNase-free DNase I solution (0.25 U/µl). Prepare a 10× DNase I reaction buffer containing 1 M NaCl, 10 mM MnCl₂ or MgCl₂, and 20 mM Tris-HCl (pH 7.0 at 25°C). Dilute this buffer to a 1× working concentration before use. Use the 1× buffer to dilute the DNase I enzyme to a final concentration of 0.25 U/µl. Alternatively, use the ready-to-use **FavorPrep™ DNase I Solution (Cat. No. FAD1209)** to simplify preparation.

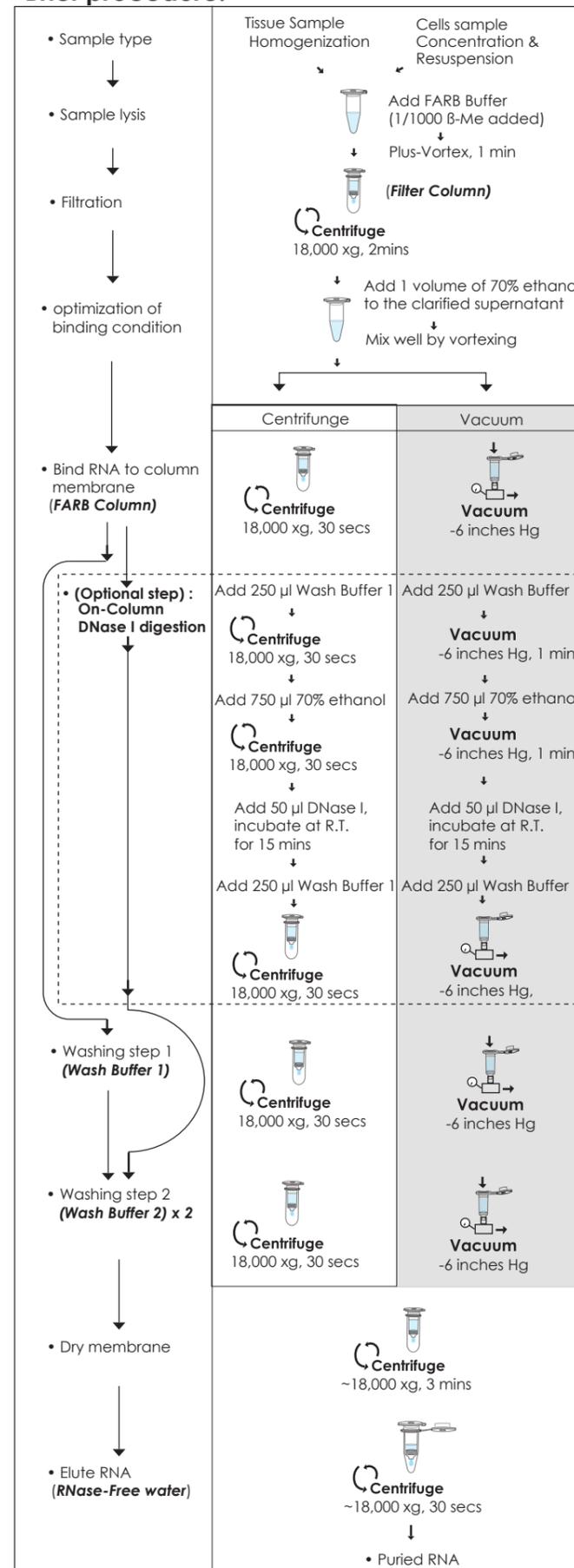
Safety Information:

CAUTION: FARB Buffers and Wash Buffer 1 contain guanidinium salts which can form highly reactive compounds when combined with bleach. **DO NOT add bleach or acidic solutions directly to the preparation waste.**

Kit Component: FARB Buffer	
Hazard contents Guanidinium thiocyanate CAS-No. 593-84-0 EC-No. 209-812-1	
GHS symbol	Warning
Hazard statement(s) H302 + H312 + H332	Harmful if swallowed, in contact with skin or if inhaled.
H314	Causes severe skin burns and eye effects.
H412	Harmful to aquatic life with long lasting effects.
Precautionary statement(s) P260	Do not breathe dust/ fume/ gas/ mist/ vapours/spray.
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection.
P301 + P312 + P330	IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell. Rinse mouth.
P303 + P361 + P353	IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
P304 + P340 + P310	IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/doctor.
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Kit Component: Wash Buffer 1	
Hazard contents Guanidine hydrochloride, 20~50%, CAS-No. 50-01-1	
GHS symbol	Warning
Hazard statement(s) H302 H319	Harmful if swallowed. Causes serious eye irritation.
Precautionary statement(s) P264 P280	Wash ... thoroughly after handling. Wear protective gloves/protective clothing/ eye protection/fac protection.
P301 + P312 + P330	IF SWALLOWED: Call a POISON CENTER/ doctor if you feel unwell. Rinse mouth.

Brief procedure:



Storage:

Kit components should be stored at room temperature (15~25°C).

Quality Control:

The quality of FavorPrep™ Tissue Total RNA Mini Kit is tested on a lot-to-lot basis according to ISO quality management system.

Specification:

Format/ Principle: spin column/silica membrane/chaotropic salt
Operation time: 30~60 mins
Length of recovery nucleic acid : >200 bp
Column Binding capacity: 100 µg RNA/column
Elution volume: 30~50 µl
Column applicability: centrifugation and vacuum

General Protocol: Animal Cells

Please Read Important Notes Before Starting Following Steps.

Additional requirement: β-Mercaptoethanol
RNase-free 70% ethanol

- Collect 1~5×10⁶ cells by centrifuging at 300 xg for 5 mins at 4°C. Remove all the supernatant. **-Note!** Do not overload, too much sample will make cell lysis incompletely and lead to lower RNA yield and purity.
- Add 350 µl of FARB Buffer and 3.5 µl of β-Mercaptoethanol to the cell pellet. Vortex vigorously for 1 min to resuspend the cells completely. **-Note:** If the clump is still visible after vortex, pipet the sample mixture up and down to break down the clump.
- Place a Filter Column to a Collection Tube, and transfer the sample mixture to the Filter Column. Centrifuge at 18,000 xg for 2 mins.
- Transfer the clarified supernatant from the Collection Tube to a new microcentrifuge tube (not provided) and measure the volume of the supernatant. Discard the Filter Column and the Collection Tube. **-Note:** Avoid to pipet any debris and pellet when transferring the supernatant.
- Add 1 volume of RNase-free 70% ethanol and mix well by vortexing.
- Place a FARB Mini Column to a Collection Tube and transfer the ethanol added sample mixture (including any precipitate) to the FARB Mini Column.
Centrifuge Centrifuge at 18,000 xg for 30 secs. Discard the flow-through and return the FARB Mini Column back to the Collection Tube.
Vacuum Apply vacuum at -6 inches Hg until the column have emptied. Switch off the vacuum and release vacuum from the manifold.
- Optional step: On-Column DNase I digestion**
Follow the steps from 7a to eliminate genomic DNA contamination. **Otherwise, proceed to step 8 directly.**
 - Add 250 µl of Wash Buffer 1 to the FARB Mini Column.
Centrifuge Centrifuge at 18,000 xg for 30 secs. Discard the flow-through and return the FARB Mini Column back to the Collection Tube.
Vacuum Apply vacuum at -6 inches Hg for 1 min. Switch off the vacuum and release vacuum from the manifold.
 - Add 750 µl of RNase-free 70% ethanol to the FARB Mini Column.
Centrifuge Centrifuge at 18,000 xg for 30 secs. Discard the flow-through and return the FARB Mini Column back to the Collection Tube.
Vacuum Apply vacuum at -6 inches Hg for 1 min. Switch off the vacuum and release vacuum from the manifold.
 - Add 60 µl of RNase-free DNase I solution (0.25 U/µl, not provided) to the membrane center of the FARB Mini Column. Place the column on the benchtop for 15 mins.
 - Add 250 µl of Wash Buffer 1 to the FARB Mini Column.
Centrifuge Centrifuge at 18,000 xg for 30 secs. Discard the flow-through and return the FARB Mini Column back to the Collection Tube.
Vacuum Apply vacuum at -6 inches Hg until the column have emptied. Switch off the vacuum and release vacuum from the manifold.
- After DNase I treatment, proceed to step 9.**
- Add 500 µl of Wash Buffer 1 to the FARB Mini Column.
Centrifuge Centrifuge at 18,000 xg for 30 secs. Discard the flow-through and return the FARB Mini Column back to the Collection Tube.
Vacuum Apply vacuum at -6 inches Hg until the column have emptied. Switch off the vacuum and release vacuum from the manifold.
- Add 750 µl of Wash Buffer 2 to the FARB Mini Column.
Centrifuge Centrifuge at 18,000 xg for 30 secs. Discard the flow-through and return the FARB Mini Column back to the Collection Tube.
Vacuum Apply vacuum at -6 inches Hg until the column have emptied. Switch off the vacuum and release vacuum from the manifold.
-Note: Make sure that ethanol has been added into Wash Buffer 2 when first use.
- Repeat step 9 for one more washing.

- Dry Column:
Centrifuge at 18,000 xg for 3 mins. Discard the flow-through and return the FARB Mini Column back to the Collection Tube.
-Important step! This step will prevent subsequent enzymatic reactions from inhibition by the residual wash buffer.
- Place the FARB Mini Column to a Elution Tube (provided).
- Add 30~50 µl of RNase-Free Water to the membrane center of the FARB Mini Column. Stand the FARB Mini Column at room temperature for 1 min. **-Important Step!** Ensure that RNase-Free Water is dispensed on the membrane center and be absorbed completely. **-Note!** : Do not use RNase-Free Water less than suggested volume (<30 µl) to elute RNA. It will lower the RNA yield.
- Centrifuge the FARB Mini Column at 18,000 xg for 30 secs to elute RNA. Store the RNA at -70°C.

Protocol: Animal Tissues

Please Read Important Notes Before Starting Following Steps.

Additional equipment: • liquid nitrogen & mortar
• a rotor-stator homogenizer, or a 20-G needle syringe.
• β-Mercaptoethanol
• RNase-free 70% ethanol

- Weight up to 30 mg of tissue sample. Grind the sample in liquid nitrogen to a fine powder with a mortar and transfer the powder to a new microcentrifuge tube (not provided). **-Note!** Avoid thawing the sample during weighing and grinding.
 - Add 350 µl of FARB Buffer and 3.5 µl of β-Mercaptoethanol. Homogenize the sample by using a rotor-stator homogenizer or by passing the sample lysate through a 20-G needle syringe 10 times. Incubate the sample at room temperature for 5 min.
-Important step: In order to release more RNA from the harder samples, it is recommended to homogenize the sample by using suitable homogenize equipment, for example, with a rotot-stator homogenizer.
 - Follow the Animal Cells Protocol starting from step 3.
- (Alternative)**
- Place up to 30 mg of tissue sample to a microcentrifuge tube. Add 350 µl of FARB Buffer and 3.5 µl of β-Mercaptoethanol. Use a provided micropistle to grind the tissue sample thoroughly.
 - Homogenize the sample by passing the ground sample through a 20-G needle syringe 10~20 times. Incubate at room temperature for 5 mins. **-Note!** For the tissue samples having low cell amount and hard to disrupt, it is recommended to proceed A1-A3 step above.
 - Follow Animal Cells Protocol starting from step 3.

Protocol: Bacteria

Please Read Important Notes Before Starting Following Steps

Additional requirement: • β-Mercaptoethanol
• RNase-free 70% ethanol
• 37°C water bath or heating block
• 2 ml screw centrifuge tube
• Prepare 100 µl lysozyme reaction solution for each reaction. Dilute lysozyme to 10 mg/ml in buffer containing 20 mM Tris-HCl, pH 8.0; 2 mM EDTA; 1.2% Triton. Alternatively, use the ready-to-use **FaverPrep™ Lysozyme Solution (Cat. No. FALZ209)** to simplify preparation.
• Acid-washed glass beads, 500~700 µm

- Transfer up to 1×10⁹ cells well-grown bacterial culture to a 2 ml screw centrifuge tube. **-Note!** Make sure the amount of total RNA harvested from sample do not exceed the column's binding capacity (100 µg) when estimate the sample size. **-Note!** Too much sample will make cell lysis incompletely and lead to lower RNA yield and purity. If RNA amount is hard to determine on some species, using ≤5×10⁸ cells as the starting sample size.
- Descend the bacterial cells at 4°C by centrifuging at 18,000 xg for 2 min. Remove all the supernatant.
- Add 100 µl of lysozyme reaction solution. Pipet up and down to resuspend the cell pellet and incubate the sample at 37°C for 10 mins.
- Add 350 µl of FARB Buffer and 3.5 µl of β-Mercaptoethanol.
- Add 250 mg of acid-washed glass beads (500~700 µm) and vortex vigorously for 5 mins to disrupt the cells.
- Centrifuge at 18,000 xg for 2 mins to spin down insoluble material. Transfer the supernatant to a microcentrifuge tube (not provided) and measure the volume of the supernatant. **-Note!** Avoid pipetting any debris and pellet in the Collection Tube.
- Follow Animal Cells Protocol starting from step 5.

Protocol: Yeast

Please Read Important Notes Before Starting Following Steps

Additional requirement: • β-Mercaptoethanol
• RNase-free 70% ethanol

- Enzymatic disruption: • Prepare 500 µl lyticase or zymolyase reaction solution for each reaction. Dilute lyticase or zymolyase to 400 U/ml in buffer containing 1 M sorbitol; 100 mM EDTA; 0.1% β-ME. Alternatively, use the ready-to-use **FaverPrep™ Lyticase Solution (Cat. No. FALC209)** to simplify preparation.
• 37°C water bath or heating block
- Mechanical disruption: • 2 ml screw centrifuge tube
• Acid-washed glass beads (500~700 µm)
- Collect up to 25 mg of yeast culture at 4°C by centrifuging at 5,000 xg for 10 mins. Remove all the supernatant.
 - Enzymtic disruption:
 - Resuspend the cell pellet in 500 µl of lyticase or zymolayse reaction solution and incubate at 37°C for 30 mins.
-Note! Prepare sorbitol buffer just before use.
 - Centrifuge at 5,000 xg for 5 mins to pellete the spheroplasts. Remove all the supernatant.
 - Add 350 µl of FARB Buffer and 3.5 µl of β-Mercaptoethanol to the pellet. Vortex vigorously to disrupt the spheroplasts for 1 min. Incubate the sample mixture at room temperature for 5 mins.
 - Mechanical disruption:
 - Add 350 µl of FARB Buffer and 3.5 µl of β-Mercaptoethanol to the pellet and vortex vigorously to resuspend the cells completely.
 - Transfer the sample mixture to a 2 ml screw centrifuge tube. Add 250 mg of acid-washed glass beads (500~700 µm) and vortex vigorously for 15 min to disrupt the cells.
 - Follow Animal Cells Protocol starting from step 5.

Protocol: paraffin-embedded tissue

Please Read Important Notes Before Starting Following Steps.

Additional equipment: • xylene & ethanol (96~100%)
• liquid nitrogen & mortar
• a rotor-stator homogenizer or a 20-G needle syringe
• β-Mercaptoethanol
• RNase-free 70% ethanol

- Transfer up to 15 mg paraffin-embedded tissue sample to a micro-centrifuge tube (not provided). **-Remove the extra paraffin to minimize the size of the sample slice.**
- Add 0.5 ml xylene, mix well and incubate at room temperature for 10 mins.
- Centrifuge at full speed for 3 min. Remove the supernatant by pipetting.
- Add 0.25 ml xylene, mix well and incubate at room temperature for 3 mins.
- Centrifuge at full speed for 3 mins. Remove the supernatant by pipetting.
- Repeat step 4 and step 5
- Add 0.3 ml ethanol (96~100%) to the deparaffined tissue, mix gently by vortexing. Incubate at room temperature for 3 mins.
- Centrifuge at full speed for 3 mins. Remove the supernatant by pipetting.
- Repeat step 7 and step 8.
- Follow Animal tissue Protocol starting from step 1 for sample disruption then follow Animal Cells protocol starting from step 3.

Protocol: RNA clean up

Please Read Important Notes Before Starting Following Steps.

Additional equipment: • ethanol (96~100%)

- Trandfer 100 µl of RNA sample to a microcentrifuge tube (not provided). -If the RNA sample is less than 100 µl, add RNase-Free Water to make the sample volume to 100 µl.
- Add 300 µl of FARB Buffer and 300 µl of RNase-free ethanol (96~100%) and mix well by vortexing.
- Place a FARB Mini Column to a Collection Tube and transfer the ethanol added sample mixture to the FARB Mini Column. Centrifuge at 18,000 xg for 1 min. Discard the flow-through and return the FARB Mini Column back to the Collection Tube.
- Follow Animal Cells Protocol starting from step 8.

Troubleshooting

- Low yield**
 - Sample not store well or thaw repeatty
 - Store samples at -80°C for long-term storage. Frozen samples do not be thawed more than once.
 - RNA Degradation
 - Harvested samples not immediately stabilized.
 - Insufficient mixing with FARB Buffer
 - Mix the sample mixture by plus-vortexing
 - Improper RNA binding condition
 - No ethanol added to the lysate (step 5) or incorrect percentage of ethanol be used.
 - Incorrect RNA elution
 - Ensure that RNase free water was added at the center of the FARB column membrane and absorbed by the membrane .
 - Incorrect preparation of Wash Buffer 2
 - Ensure that the correct volume of ethanol (96~100%) was added to Wash Buffer 2 when first use.
- Eluted RNA does not perform well**
 - Residual ethanol contamination
 - Ensure that FARB Column has done centrifugation for an additional 3 mins at speed 18,000 xg (step 11) after washing step.

Product category of Favorgen:

For more information please visit Favorgen web site www.favorgen.com

Nucleic Acid Extraction - spin column (silica membrane)
• Viral DNA/RNA Mini Kit
• Viral Nucleic Acid Extraction Mini Kit
• Circulating Nucleic Acid Extraction Mini Kit

RNA Extraction - spin column (silica membrane)
• Blood/Cultured Cells Total RNA Extraction Mini/Maxi Kit
• Tissue Total RNA Extraction Mini/Maxi Kit
• Plant Total RNA Extraction Mini/Maxi Kit
• RNA Clean Up Mini Kit

DNA Clean-Up - spin column (silica membrane)
• PCR Clean Up Mini Kit
• Gel Purification Mini Kit
• Gel/PCR Purification Mini Kit
• Gel/PCR Purification Micro Kit

DNA Extraction - spin column (silica membrane)
• Blood Genomic DNA Extraction Mini/Midi/Maxi Kit
• Plant Genomic DNA Extraction Mini/Maxi Kit
• Food DNA Extraction Mini Kit
• Milk Bacterial DNA Extraction Mini Kit
• Tissue Genomic DNA Extraction Mini Kit
• FFPE Tissue DNA Extraction Micro Kit
• Fungi/Yeast Genomic DNA Extraction Mini Kit
• Soil DNA Extraction Mini Kit
• Stool DNA Extraction Mini Kit

Extraction Reagent
• Tri-RNA Reagent (Acid Guanidinium Thiocyanate-Phenol-Chloroform Extraction)

High Efficiency (HE) - spin column (silica membrane)
• Tissue Genomic DNA Extraction HE Mini Kit
• Blood Genomic DNA Extraction HE Mini Kit
• Soil DNA Extraction HE Mini Kit
• Stool DNA Extraction HE Mini Kit
• Bacterial Genomic DNA Extraction HE Mini Kit
• Urine DNA Extraction HE Mini Kit
• Gel/PCR Purification HE Mini Kit
• Bacterial RNA Extraction HE Mini Kit
• Circulating Nucleic Acid Extraction HE Mini Kit

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