FastGene®

FastGene® RNA Basic Kit

FastGene® RNA Premium Kit

For purification of total RNA from cultured cells and tissues

High Yield, High Purity

FastGene® RNA Basic Kit

Cat.No.: FG-80006, 6 preparations
Cat.No.: FG-80050, 50 preparations
Cat.No.: FG-80250, 250 preparations

FastGene® RNA Premium Kit

Cat.No.: FG-81006, 6 preparations
Cat.No.: FG-81050, 50 preparations
Cat.No.: FG-81250, 250 preparations

FastGene® RNA Isolation Kits are intended for research use only. Not for use in clinical diagnostics.

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Kit Contents

FastGene® RNA Basic Kit

Cat. No. FG-80006, FG-80050, FG-80250

Cat. No.: FG-80006 (6 preps)	
Lysis buffer (RL)	4 ml
Wash buffer 1 (RW1)	4 ml
Wash buffer 2 (RW2)	1 ml
Elution buffer (RE: RNase free water)	1.5 ml
FastGene® RNA binding column (green, with collection tubes)	6
1.5 ml collection tubes	6
2 ml collection tubes	12
User manual	1
Cat. No.: FG-80050 (50 preps/kit)	
Lysis buffer (RL)	25 ml
Wash buffer 1 (RW1)	35 ml
Wash buffer 2 (RW2)	10 ml
Elution buffer (RE: RNase free water)	15 ml
FastGene® RNA binding column (green, with collection tubes)	50
1.5 ml collection tubes	50
2 ml collection tubes	100
User manual	1
Cat. No.: FG-80250 (250 preps/kit)	
Lysis buffer (RL)	125 ml
Wash buffer 1 (RW1)	170 ml
Wash buffer 2 (RW2)	50 ml
Elution buffer (RE: RNase free water)	100 ml
FastGene® RNA binding column (green, with collection tubes)	250
1.5 ml collection tubes	250
2 ml collection tubes	500
User manual	1

FastGene® RNA Premium Kit
Cat. No. FG-81006, FG-81050, FG-81250

Cat. No.: FG-81006 (6 preps)	
Lysis buffer (RL)	4 ml
Wash buffer 1 (RW1)	4 ml
Wash buffer 2 (RW2)	2 ml
RNA re-binding buffer (RBD)	1 ml
Elution buffer (RE: RNase free water)	1.5 ml
DNase I reconstitution solution	1.5 ml
10 x DNase I reaction buffer	50 µl
DNase I (lyophilized)	110 Kunitz units
FastGene® RNA filter column (yellow, with collection tubes)	6
FastGene® RNA binding column (green, with collection tubes)	6
FastGene® RNA mini-elute column (neutral, with collection tubes)	6
1.5 ml collection tubes	12
2 ml collection tubes	18
User manual	1
Cat. No.: FG-81050 (50 preps/kit)	
Lysis buffer (RL)	25 ml
Wash buffer 1 (RW1)	35 ml
Wash buffer 2 (RW2)	20 ml
RNA re-binding buffer (RBD)	8 ml
Elution buffer (RE: RNase free water)	30 ml
DNase I reconstitution solution	1.5 ml
10 x DNase I reaction buffer	500 µl
DNase I (lyophilized)	110 Kunitz units
FastGene® RNA filter column (yellow, with collection tubes)	50
FastGene® RNA binding column (green, with collection tubes)	50
FastGene® RNA mini-elute column (neutral, with collection tubes)	50
1.5 ml collection tubes	100
2 ml collection tubes	150
User manual	1
Cat. No.: FG-81250 (250 preps/kit)	
Lysis buffer (RL)	125 ml
First wash buffer (RW1)	170 ml
Second wash buffer (RW2)	2 x 50 ml
RNA re-binding buffer (RBD)	36 ml
Elution buffer (RE: nuclease free water)	200 ml
DNase I reconstitution solution	1.5 ml
10 x DNase I reaction buffer	2 x 1 ml
DNase I (lyophilized)	560 Kunitz units
FastGene® RNA filter column (yellow, with collection tubes)	250
FastGene® RNA binding column (green, with collection tubes)	250
FastGene® mini-elute column (neutral, with collection tubes)	250
1.5 ml collection tubes	500
2 ml collection tubes	750
User manual	1

IMPORTANT NOTICE for FastGene® RNA Premium kit:

Upon receipt of FastGene® RNA Premium kit store the FastGene® mini-elute column (neutral color) at 2-8°C!

Storage and Stability

Store the FastGene® RNA Basic/Premium kit at room temperature (15-25 °C). Under these conditions the FastGene® RNA Basic/Premium kit components are guaranteed for 15 month after manufacture. However, store the FastGene® RNA mini-elute columns (neutral color) immediately upon receipt at 2-8 °C. Storing FastGene® RNA mini-elute column at room temperature will reduce performance.

Reagents/Material to be supplied by user

- Reducing agent: DTT or TCEP or 2-Mercaptoethanol (2-ME)
- Freshly prepared 70 % ethanol
- 96-100 % ethanol
- Sterile Gloves
- Sterile, RNase-free pipet tips
- 1.5 ml reaction tubes
- Equipment: pipette, centrifuge, heat block, vortex mixer, homogenizer

Safety Information

The following components of the kit contain hazardous contents. Wear gloves and goggles and follow the safety instructions given in this section.

1. GHS Classification (Hazard and Precaution Phrases)

Only harmful features do not need to be labeled with H and P phrases up to 125 mL or 125 g.

Component of the kit*	Hazardous content	GHS Symbol	Hazard Phrases	Precaution Phrases
Buffer RL	*Guanidinium thiocyanate 30-50%*	 DANGER	H302; H314; H412	P280; P303+P361+P353; P305+P351+P338; P310
Buffer RW1	*Guanidinium hydrochlorid 5-15%*	 DANGER	H225; H315; H319	P210; P280
Buffer RBD	*Guanidinium thiocyanate 5-15%*	 DANGER	H302; H314; H412	P280; P303+P361+P353; P305+P351+P338; P310

**Bottles with a volume less than 125 ml will only show a simplified labelling.*

Hazard Phrases

H225 *Highly flammable liquid and vapor.*
H302 *Harmful if swallowed.*
H314 *Causes server skin burns and eye damage.*
H315 *Causes skin irritation.*
H319 *Causes serious eye irritation.*
H334 *May cause allergy or asthma symptoms or breathing difficulties if inhaled.*
H412 *Harmful to aquatic life with long lasting effects.*

Precaution Phrases

P210 *Keep away from heat/sparks/open flames/hot surfaces. - No smoking.*
P280 *Wear protective gloves/protective clothing/eye protection/face protection.*
P303+P363+P353 *IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.*
P305+P351+P338 *IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.*
P310 *Immediately call a POISON CENTER or doctor/physician.*

Description of FastGene® RNA Basic/Premium Kits

Specification

Sample volume	< 5 x 10^6 – 1 x 10^7 cultured cells < 10-20 mg animal tissues
Typical RNA yield	10-20 µg from 1 x 10^6 HeLa cells 50-100 µg from 20 mg liver mice tissue
Average operation time	40 min/6 preps (Basic Kit) 60 min/6 preps (Premium Kit)
Elution volume	50 µl (Basic Kit) 10-50 µl (Premium Kit)

Principle

The FastGene® RNA **Basic** Kit purifies total RNA samples from mammalian tissues and cultured cells. The isolated RNA can be used for a variety of downstream applications e.g. RT-PCR, qPCR, cDNA synthesis, northern blot, next generation sequencing and much more. In order to prevent RNA degradation the sample is treated right at the beginning with an RNases inhibitory lysis buffer RL. This step ensures purification of intact RNA. Addition of ethanol provides appropriate RNA binding conditions to the silica membrane of the FastGene® binding column. In following steps contaminations are efficiently washed away with the supplied buffers RW1 and RW2 from the column. High-quality RNA is subsequently eluted in 50 µl RE buffer. The purified RNA is ready for downstream applications or can be stored at -70 °C in a freezer.
In general, the selective RNA binding silica membrane of the FastGene® RNA Basic Kit efficiently removes most of the DNA without DNase I treatment. But it cannot be completely excluded that a tiny amount of gDNA remains in eluted RNA solution. Some very DNA sensitive downstream RNA applications could need further DNA removal. In order to ensure the efficient removal of gDNA, additional steps are necessary. The FastGene® RNA **Premium** Kit guarantees due to an optimized DNase I treatment in combination with a specifically engineered FastGene® mini-elute column technology pure high-quality RNA. Unlike in kits of other suppliers the DNase I treatment will take place in liquids and not on top of the column membrane. This increases the DNase I efficiency a lot. Following RNA is bound to the membrane of the FastGene® mini-elute column that possesses a high RNA binding capacity. A much higher RNA concentration can be reached due to the small column diameter, so that elution volume can be decreased to 10 µl.

Sample Preparation

RNA is not protected against digestion until the sample material is flash frozen or disrupted in the presence of RNase inhibiting or denaturing agents. Therefore it is important that samples are flash frozen in liquid N2 immediately and stored at -70 °C or processed as soon as possible with the FastGene® RNA kit.

Cultured animal cells are collected by centrifugation and directly lysed by adding lysis buffer RL1 according to the protocol (step 2). Make sure that the cell culture medium is removed completely before adding lysis buffer RL1.

Animal tissues are often solid and must therefore be broken up mechanically as well as lysed. It is essential for efficient RNA preparation that all the RNA contained in the sample is released from the cells by disruption.
The most commonly used technique for disruption of animal tissues is grinding with a pestle and mortar or using the FastGene® Mixy Professional tissue grinder (NG010, see ordering information). Grind the sample to a fine powder in the presence of liquid N2. Take care that the sample does not thaw during or after grinding or weighing and add the frozen powder to an appropriate aliquot of lysis buffer RL1 containing reducing agent (see chapter *preparation of working solutions*)

Depending on the amount of starting material, the viscosity of the lysed sample has to be reduced further for optimal results by passing the lysed sample > 10 times through a 0.9 mm syringe needle or by using the FastGene® Filter columns included in the Premium kit.

Make sure not to use higher amount of starting material since that can decrease yield and purity of the eluated RNA.

RNA quantification, quality and storage

We recommend to determine the quantity and quality of isolated RNA to ensure best conditions for every downstream application. The easiest way to determine the concentration and purity of isolated RNA is to measure the absorbance at 260 nm and 280 nm with a spectrophotometer. 40 µg of RNA/ml corresponds to 1 O.D. unit measured at 260 nm. For spectrophotometric analysis it is advisable to dilute the sample in a buffered solution, e.g. TE (Tris EDTA) buffer. Due to the DEPC treatment the RE buffer is slightly acidic and can cause a decrease of absorbance values, so it is not recommended to measure RNA absorbance with RE buffer. Pure nucleic acids have an A_{260}/A_{280} ratio of 2.0 and pure proteins one of 0.6. On that account a ratio value of 1.8-2.0 represents 90-100% pure nucleic acid.

RNA quality can be also assessed by electrophoresis analysis. In optimum case for eukaryotes two distinct bands should appear on the gel – the 28S and 18S (23S and 16S for bacteria) ribosomal RNA bands. Degradation during preparation, handling or storage results in a smear towards lower molecular weight sized RNAs.

To ensure RNA stability keep RNA frozen at -20°C for short-term or -70°C for long-term storage.

Preparation of working solutions

Lysis buffer RL

Add one of the below listed reducing agents to buffer RL only at the following ratio according to the number of samples.

1. Final concentration of DTT: 40 mM
2. Final concentration of TCEP-HCl: 20 mM
3. Final concentration of 2-Mercaptoethanol: 1% (v/v)*

Reductant	Volume of reductant	Volume of Buffer RL	Final concentration of reductant
2 M DTT	20 µl	1 ml	40 mM
1 M TCEP	20 µl	1 ml	20 mM
2-ME	10 µl	1 ml	1%*

* 2-ME is generally sold with a concentration of 14.3 M, the final concentration of 1% is 143 mM in terms of molar concentration.

Second wash buffer RW2 (Basic Kit)

6 preps	50 preps	250 preps
Add 4 ml ethanol* to 1 ml RW2 and mix	Add 40 ml ethanol* to 10 ml RW2 and mix	Add 200 ml ethanol* to 50 ml RW2 and mix

*96 – 100% ethanol

Second wash buffer RW2 (Premium Kit)

6 preps	50 preps	250 preps
Add 8 ml ethanol* to 2 ml RW2 and mix	Add 80 ml ethanol* to 20 ml RW2 and mix	Add 200 ml ethanol* to 50 ml RW2 and mix

*96 – 100% ethanol

Lyophilized DNase I (only Premium Kit)

6 preps	50 preps	250 preps
Add 55 µl DNase I reconstitution solution to a tube of lyophilized DNase I	Add 55 µl DNase I reconstitution solution to a tube of lyophilized DNase I	Add 280 µl DNase I reconstitution solution to a tube of lyophilized DNase I

In order to collect the DNase on the bottom of the vial spin down the powder by using a centrifuge before opening the tube. Add the indicated volume of the DNase I reconstitute solution, mix gently by tapping the tube. Do not vortex DNase! Dissolved DNase I can be stored in aliquoted tubes at -20 ˚C. We do not recommend to refreeze and thaw the enzyme.

RNA re-binding buffer RBD (only Premium Kit)

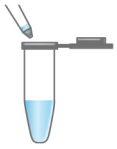
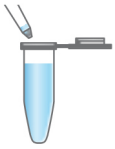
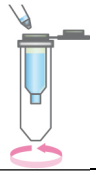
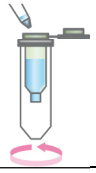
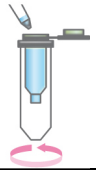
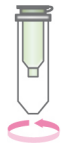
6 preps	50 preps	250 preps
Add 1 ml ethanol* to 1 ml RBD	Add 7 ml ethanol* to 8 ml RBD	Add 34 ml ethanol* to 36 ml RBD

*96 – 100% ethanol

Total RNA purification protocol

I. FastGene® RNA Basic Kit Quick protocol

Before starting the purification, please ensure that buffer RL, buffer RW2. Buffer RBD and DNase I are prepared accordingly (see chapter “Preparation of working solutions”).

Step	Standard protocol	Large input protocol
1. Sample quantity	< 5×10^6 cultured animal cells < 10 mg animal tissues	< 1 x 10^7 cultured animal cells < 20 mg animal tissues
2. Resuspension/ homogenisation by cell lysis	 350 µl buffer RL (with final concentration of 20 mM DTT or TCEP)	 600 µl buffer RL (with final concentration of 20 mM DTT or TCEP)
3. Optimize RNA binding conditions	 Add 350 µl 70 % ethanol Mix thoroughly	 Add 600 µl 70 % ethanol Mix thoroughly
4. RNA binding	 Load up to 700 µl mix onto FastGene® RNA binding column (green) Centrifuge at ≥ 10,000 x g 1 min at RT (Repeat that step till whole sample solution is loaded)	
5. Protein elimination	 Add 600 µl of buffer RW1 Centrifuge at ≥ 10,000 x g 30 s at RT Transfer column in new 2.0 ml collection tube	
6. Desalination	 Add 700 µl of buffer RW2 Centrifuge at ≥ 10,000 x g 30 s at RT	
7. Removal of RW2	 Centrifuge at full speed 1 min at RT Transfer spin column to new 1.5 ml collection tube	
8. Elution of RNA	 Add 50 µl of buffer RE to membrane center Centrifuge at ≥ 10,000 x g 1 min at RT	

II. FastGene® RNA Basic Kit detailed protocol

Before starting the purification, please ensure that the following preparations have been made (see chapter “Preparation of working solutions”):

- Reductant is added to lysis buffer RL
- Addition of ethanol to buffer RW2

1. Harvest samples in a tube (not provided in the kit). Proceed the next step as quickly as possible.

	Standard	Large input
Cells	~5 x 10^6	~1 x 10^7
Tissue	~10 mg	~20 mg

2. Add buffer RL to the sample
Make sure that reducing agents are added to buffer RL (see chapter “Preparation of working solutions)

	Standard	Large input
Quantity of buffer RL	350 µl	600 µl

3. Add ethanol (70 % v/v) to the lysate and mix well by pipetting.

	Standard	Large input
Quantity of ethanol	350 µl	600 µl

For the subsequent steps both “Standard” and “Large Input” are the same operation.

4. Take a FastGene® RNA binding column (green) placed in a collection tube. Load up to 700 µl of the mixture into the FastGene® RNA binding column and centrifuge ≥10,000 x g for 1 min at room temperature (20-25°C).
For large input, discard the flow-through and repeat this step until no more lysate is available.

5. Add 600 µl of buffer RW1 and centrifuge at ≥ 10,000 x g for 30 s at room temperature (20-25°C), discard the flow-through and re-insert the spin column to a new 2 ml collection tube.

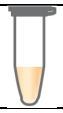
6. Add 700 µl of buffer RW2* and centrifuge at ≥ 10,000 x g for 30 s at room temperature (20-25°C), discard the flow-through and re-insert the spin column to a new 2 ml collection tube.
**Make sure that ethanol is added to buffer RW2 (see chapter “Preparation of working solutions”).*

7. Centrifuge at full speed for 1 min at room temperature (20-25°C) to remove residual buffer RW2.
Transfer spin column to a new 1.5 ml collection tube.

8. Add 50 µl of buffer RE to the center of the membrane in the FastGene® RNA binding column. Centrifuge at ≥ 10,000 x g for 1 min at room temperature (20-25°C) in order to elute the purified RNA.

III. FastGene® RNA Premium Kit Quick protocol

Before starting the purification, please ensure that buffer RL, buffer RW2. Buffer RBD and DNase I are prepared accordingly (see chapter “Preparation of working solutions”).

Step	Standard protocol	Large input protocol
1. **Sample quantity**	< 5 x 10^6 cultured animal cells < 10 mg animal tissues	< 1 x 10^7 cultured animal cells < 20 mg animal tissues
2. **Resuspension/ lysis of the cells**	 350 µl buffer RL (with final concentration of 20 mM DTT or TCEP)	 600 µl buffer RL (with final concentration of 20 mM DTT or TCEP)
3. **Filtration of cellular debris**	 Transfer lysate into a FastGene® RNA filter column (yellow) Centrifuge at ≥ 10,000 x g 1 min at RT	
4. **Optimize RNA binding conditions**	 Add 350 µl 70 % ethanol Mix thoroughly	 Add 600 µl 70 % ethanol Mix thoroughly
5. **RNA binding**	 Load up to 700 µl mix onto FastGene® RNA binding column (green) Centrifuge at ≥ 10,000 x g; 1 min at RT	
6. **Protein elimination**	 Add 600 µl of buffer RW1 Centrifuge at ≥ 10,000 x g 30 s at RT	
7. **Desalination**	 Add 700 µl of buffer RW2 Centrifuge at ≥ 10,000 x g 30 s at RT	
8. **Removal of RW2**	 Centrifuge at full speed 1 min at RT Transfer spin column to new 1.5 ml collection tube	
9. **Elution of RNA**	 Add 50 µl of buffer RE to membrane center Centrifuge at ≥ 10,000 x g 1 min at RT	
10. **Optimize DNase I conditions**	 Add 5 µl 10 x DNase I reaction buffer to the eluate	
11. **DNA digestion**	 Add 1 µl of DNase I to the mixture Mix by pipetting Incubate for 10 min at RT	
12. **RNA rebinding optimization**	 Add 250 µl of buffer RBD to the mixture Mix thoroughly by pipetting	
13. **RNA binding**	 Transfer mixture into FastGene® RNA mini-elute column (neutral) Centrifuge at ≥ 10,000 x g; 1 min at RT	
14. **Desalination/ Elimination of digested DNA**	 Add 700 µl buffer RW2 Centrifuge at ≥ 10,000 x g 30 s at RT Transfer spin column in new 2 ml collection tube	
15. **Removal of RW2**	 Centrifuge at full speed 1 min at RT Transfer spin column in new 1.5 ml collection tube	
16. **Elution of RNA**	 Add 10 – 50 µl of buffer RE to the membrane center Centrifuge at ≥ 10,000 x g 1 min at RT	

IV. FastGene® RNA Premium Kit detailed protocol

Before starting the purification, please ensure that the following preparations have been made (see chapter “Preparation of working solutions”):

- Reductant is added to lysis buffer RL
- Addition of ethanol to buffer RW2
- Addition of ethanol to buffer RBD
- Reconstitution of DNase I

1. Harvest samples in a reaction tube (not provided in the kit). Proceed the next step as quickly as possible.

	Standard	Large input
Cells	~5 x 10^6	~1 x 10^7
Tissue	~10 mg	~20 mg

2. Add buffer RL to the sample
Make sure that reducing agents are added to buffer RL (see chapter “Preparation of working solutions)

	Standard	Large input
Quantity of buffer RL	350 µl	600 µl

3. Take a FastGene® RNA filter column (yellow) placed in a collection tube. Transfer lysate into a FastGene® RNA filter column and centrifuge at ≥ 10,000 x g for 1 min at room temperature.

4. Add ethanol (70 % v/v) to the lysate and mix well by pipetting.

	Standard	Large input
Quantity of ethanol	350 µl	600 µl

For the subsequent steps both “Standard” and “Large Input” are the same operation.

5. Take a FastGene® RNA binding column (green) placed in a collection tube. Apply up to 700 µl of the mixture into a FastGene® RNA binding column and centrifuge at ≥ 10,000 x g for 1 min at room temperature (20-25°C). For large input, discard the flow-through and repeat this step until no more lysate is available.

6. Add 600 µl of buffer RW1 and centrifuge at ≥ 10,000 x g for 30 s at room temperature (20-25°C), discard the flow-through and re-insert the spin column to a new 2 ml collection tube.

7. Add 700 µl of buffer RW2* and centrifuge at ≥ 10,000 x g for 30 s at room temperature (20-25°C), discard the flow-through and re-insert the spin column to a new 2 ml collection tube.
**Make sure that ethanol is added to buffer RW2 (see chapter “Preparation of working solutions”).*

8. Centrifuge at full speed for 1 min at room temperature (20-25°C) to remove residual buffer RW2. Transfer FastGene® RNA binding column to a new 1.5 ml collection tube.

9. Add 50 µl of buffer RE to the center of the membrane of the FastGene® RNA binding column. Centrifuge at ≥ 10,000 x g for 1 min at room temperature (20-25°C) in order to elute the purified RNA.

10. Add 5 µl of 10 x DNase I reaction buffer to the 50 µl of the eluted sample and mix well by pipetting.

11. Add 1 µl of DNase I enzyme solution* to the mixture, mix thoroughly by pipetting and incubate for 10 minutes at room temperature (20-25°C).
** Please prepare DNase I solution before (see chapter "Preparations of working solutions").*

12. Add 250 µl of buffer RBD* to the DNase I treated mixture and mix well by pipetting.
**Make sure that ethanol is added to buffer RBD (see chapter "Preparation of working solutions").*

13. Take a FastGene® RNA mini-elute column (neutral) placed in a collection tube. Apply all of the mixture into the FastGene® RNA mini-elute column and centrifuge at ≥ 10,000 x g for 1 min at room temperatur (20-25°C).

14. Apply 700 µl of buffer RW2 into the FastGene® RNA mini-elute column and centrifuge at ≥ 10,000 x g for 30 s at room temperature (20-25°C), discard the flow-through and re-insert the FastGene® RNA mini-elute column to a new 2 ml collection tube.

15. Centrifuge at full speed for 1 min at room temperature to remove residual buffer RW2. Transfer FastGene® RNA mini-elute column to a new 1.5 ml collection tube.

16. Add 10-50 µl of buffer RE to the center of the membrane in the FastGene® RNA mini-elute column. Centrifuge at ≥ 10,000 x g for 1 min at room temperature (20-25°C).

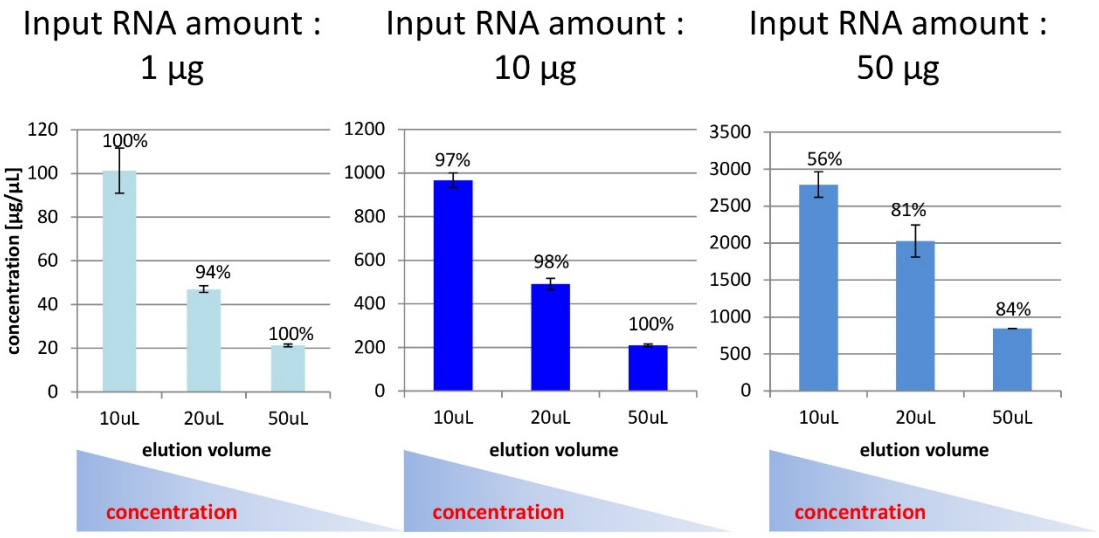


Figure 1 shows the correlation between amount of starting material, elution volume, RNA yield and RNA concentration.

Troubleshooting

Problem	Possible cause	Suggestions
No or low RNA concentration	Too small amount of starting material	Increasing of starting material up to the material specific recommended amount
	Immoderate amount of starting material	Reduction of starting material to the material specific recommended amount
	Insufficient homogenization or disruption of starting material	Increase incubation time with the lysis buffer
	Incomplete elution of RNA from spin column membrane	Repeat elution step with a prior warming of the RNase free elution water to 60 °C
	Incorrect DNase I reaction mixture	Be sure to comply with the instruction
Filter column is blocked	Immoderate amount of starting material	Reduction of starting material to the material specific recommended amount
	Too small amount of starting material	Increasing of starting material up to the recommended amount
	Insufficient homogenization or disruption of starting material	Complete homogenising of starting material and increasing of centrifugation time
RNA degradation	RNase contamination	Decontamination of all by user supplied plastics, reagents and work equipment
Low A_{260}/A_{230} ratio	Acidic buffer or water used for RNA dilution	As DEPC treated water becomes weakly acidic and decreases the absorbance value, please use TE buffer etc.
	Amount of sample material is too high	If the sample amount is too high, impurities could lead to a clogged membrane. Co-purified proteins or DNA can change the OD ratio.
	For cultured cells: Medium was not removed efficiently from cultured cells	Please completely remove the medium from the cell pellet. Residual medium leads to insufficient lysis procedure.
	Incomplete DNase I digest	Increasing of DNase I incubation time

DNA contamination	Immoderate amount of starting material	Reduction of starting material to the material specific recommended amount
	Incorrect DNase I reaction mixture	Be sure to comply with the instruction
Suboptimal performance in downstream applications	Salt contamination	Before drying the membrane by centrifugation, please use a new collection tube. In some cases it may be good to repeat washing step with the second wash buffer (RW2). RW2 must have room temperature.
	Incorrect storage of RNA	Keep diluted RNA on ice and store RNA for long term at -70 °C or colder.
	Residual ethanol	After washing with buffer RW2, dry the membrane according to the protocol. ① When you remove the column, please make sure that the column is not in touch with the liquid inside the collection tube.. ② Add the elution buffer RE to the center of the membrane. Carryover of Ethanol will affect downstream applications.

Ordering Information

Cat. No.	Product	Content
FG-80006	FastGene® RNA Basic Kit	6 preparations
FG-80050	FastGene® RNA Basic Kit	50 preparations
FG-80250	FastGene® RNA Basic Kit	250 preparations
FG-80RL025	FastGene® RNA Lysis Buffer	25 ml
FG-80RL125	FastGene® RNA Lysis Buffer	125 ml
FG-81006	FastGene® RNA Premium Kit	6 preparations
FG-81050	FastGene® RNA Premium Kit	50 preparations
FG-81250	FastGene® RNA Premium Kit	250 preparations
NG010	Tissue Grinder Mixy Professional	1
NG006	Pestles for Tissue Grinder Mixy Professional	100

Contact Information

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JAPAN

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