Version: 1.2

# 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<br>< 10-20 mg animal tissues |
| Typical RNA yield | 10-20 µg from 1 x $10^6$ HeLa cells<br>50-100 µg from 20 mg liver mice tissue |
| Average operation time | 40 min/6 preps (Basic Kit)<br>60 min/6 preps (Premium Kit) |
| Elution volume | 50 µl (Basic Kit)<br>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)

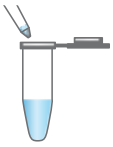
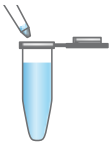
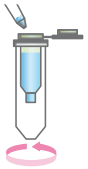
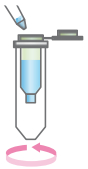
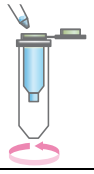
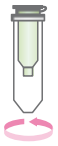
| 6 preps | 50 preps | 250 preps |
|---|---|---|
| Add 1 ml ethanol* to 1 ml RBD | Add 7 ml ethanol* to 8 ml RBD | Add 34 ml ethanol* to 36 ml RBD |

*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** | < $5\times10^6$ cultured animal cells<br>< 10 mg animal tissues | < 1 x $10^7$ cultured animal cells<br>< 20 mg animal tissues |
| **2. Resuspension/ homogenisation by cell lysis** |  350 µl buffer RL (with final concentration of 40 mM DTT or 20 mM TCEP) |  600 µl buffer RL (with final concentration of 40 mM DTT or 20 mM TCEP) |
| **3. Optimize RNA binding conditions** |  Add 350 µl 70 % ethanol<br>Mix thoroughly |  Add 600 µl 70 % ethanol<br>Mix thoroughly |
| **4. RNA binding** |  Load up to 700 µl mix onto FastGene® RNA binding column (green)<br>Centrifuge at ≥ 10,000 x g<br>1 min at RT<br>(Repeat that step till whole sample solution is loaded) | |
| **5. Protein elimination** |  Add 600 µl of buffer RW1<br>Centrifuge at ≥ 10,000 x g<br>30 s at RT<br>Transfer column in new 2.0 ml collection tube | |
| **6. Desalination** |  Add 700 µl of buffer RW2<br>Centrifuge at ≥ 10,000 x g<br>30 s at RT | |
| **7. Removal of RW2** |  Centrifuge at full speed<br>1 min at RT<br>Transfer spin column to new 1.5 ml collection tube | |
| **8. Elution of RNA** |  Add 50 µl of buffer RE to membrane center<br>Centrifuge at ≥ 10,000 x g<br>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.

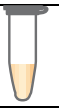
6. Add 700 µl of buffer RW2* and centrifuge at ≥ 10,000 x g for 30 s at room temperature (20-25°C), discard the flow-through and re-insert the spin column to a new 2 ml collection tube.
**Make sure that ethanol is added to buffer RW2 (see chapter “Preparation of working solutions”).*

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<br>< 10 mg animal tissues | < 1 x $10^7$ cultured animal cells<br>< 20 mg animal tissues |
| **2. Resuspension/ lysis of the cells** |  350 µl buffer RL (with final concentration of 40 mM DTT or 20 mM TCEP) |  600 µl buffer RL (with final concentration of 40 mM DTT or 20 mM TCEP) |
| **3. Filtration of cellular debris** |  Transfer lysate into a FastGene® RNA filter column (yellow)<br>Centrifuge at ≥ 10,000 x g<br>1 min at RT | |
| **4. Optimize RNA binding conditions** |  Add 350 µl 70 % ethanol<br>Mix thoroughly |  Add 600 µl 70 % ethanol<br>Mix thoroughly |
| **5. RNA binding** |  Load up to 700 µl mix onto<br>FastGene® RNA binding column (green)<br>Centrifuge at ≥ 10,000 x g; 1 min at RT | |
| **6. Protein elimination** |  Add 600 µl of buffer RW1<br>Centrifuge at ≥ 10,000 x g<br>30 s at RT | |
| **7. Desalination** |  Add 700 µl of buffer RW2<br>Centrifuge at ≥ 10,000 x g<br>30 s at RT | |
| **8. Removal of RW2** |  Centrifuge at full speed<br>1 min at RT<br>Transfer spin column to new 1.5 ml collection tube | |
| **9. Elution of RNA** |  Add 50 µl of buffer RE to membrane center<br>Centrifuge at ≥ 10,000 x g<br>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<br>Mix by pipetting<br>Incubate for 10 min at RT | |
| **12. RNA rebinding optimization** |  Add 250 µl of buffer RBD to the mixture<br>Mix thoroughly by pipetting | |
| **13. RNA binding** |  Transfer mixture into<br>FastGene® RNA mini-elute column (neutral)<br>Centrifuge at ≥ 10,000 x g; 1 min at RT | |
| **14. Desalination/ Elimination of digested DNA** |  Add 700 µl buffer RW2<br>Centrifuge at ≥ 10,000 x g<br>30 s at RT<br>Transfer spin column in new 2 ml collection tube | |
| **15. Removal of RW2** |  Centrifuge at full speed<br>1 min at RT<br>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<br>Centrifuge at ≥ 10,000 x g<br>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).

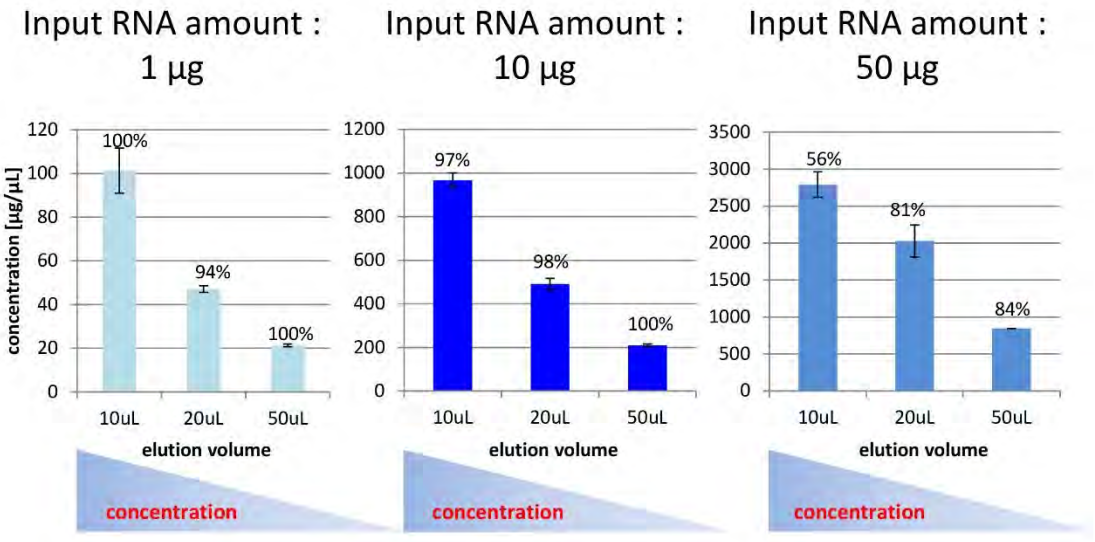



Figure 1 shows the correlation between amount of starting material, elution volume, RNA yield and RNA concentration.

# 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:<br>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.<br>① When you remove the column, please make sure that the column is not in touch with the liquid inside the collection tube..<br>② Add the elution buffer RE to the center of the membrane.<br>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 |

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