

Magtration System 6GC

Automated Nucleic Acid Isolation and Purification System

Quick Manual

~ RNA Purification ~



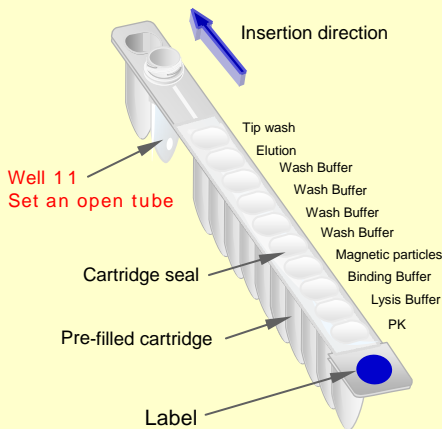
Operation Time

RNA extraction from cells or tissue samples : Approx. 35min (Without DNase treatment)
Approx. 45min (Including DNase treatment)



Reagents (GC Series Magtration® - MagaZorb® RNA Common Kit 200 : Code No. E2004)
IC card (Magtration® - MagaZorb® RNA / Common : Code No. I-6054)

Check! !



1. The sample (up to 10 mg of tissue sample or 10^6 cells pellet) is placed into a 1.5ml screw cap tube (supplied with the kit). Homogenized tissue is combined with 150 μ l of a RNA stabilizer (e.g., RNA^{later}¹) in a 1.5ml tube for a tissue sample. For a cell sample, the cells are suspended homogeneously in 200 μ l of physiological saline by pipetting, etc.
- * A soft tissue sample can be crushed with a pestle or similar item. A hard tissue sample can be crushed with a tissue crusher (e.g., cryoshocker, beads shocker, etc.) after being combined with RNA^{later}[®]. Before loading the sample on the Magtration[®] System, ensure that no solid tissue remains in the sample.
2. The tube containing the crushed sample is loaded in the prescribed location in the Tip rack.
3. If DNase treatment is needed, 100 μ l of DNase solution in a 1.5ml tube (approximately 40 units)² is loaded on the tip rack.
4. If bubbles are seen in the reagent cartridge, remove them by tapping the cartridge. Bubbles in the cartridge may disrupt complete aspiration, and may produce more bubbles by mixing. When the droplets of reagent are presented on the seal or on the side wall of well, remove them by shaking gently. In case of finding magnetic particles on the side wall, tilt the cartridge and agitate it gently to remove the particles from the wall before loading.

¹ RNA^{later}[®] is the trademark of Ambion Inc.



Preparation of Sample and Supplies

Sample : Cell suspension (200 μ l) or Homogenized tissue solution (150 μ l)

DNase solution 100 μ l
(in a 1.5 ml tube supplied with the kit)
* If DNase treatment is needed.

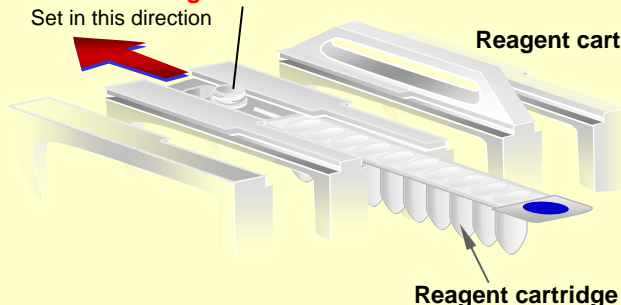
Tip and Tip holder
(supplied with the Kit)

Screw cap tube
(in a 1.5 ml tube supplied with the kit)
for collection of the eluate

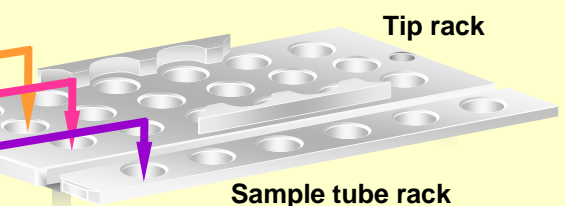
The 1.5 ml tube is set in after the reagent cartridge is loaded on the rack.

Set in this direction

Reagent cartridge rack



Reagent cartridge



2 DNase solution: If Ambion DNase is used, a mixture of 20 μ l of DNase solution and 80 μ l of 10x DNase buffer (contained in a 1.5 ml screw cap tube) is loaded.

* Follow the setting direction, Insert the Reagent cartridge in the Reagent cartridge rack until it sounds "click".



Start of Processing

Check if the IC card is set.
(6GC : Code No. I-6054) ()

Turn on the power of Magtration System 6GC.
Starting menu is displayed on LCD automatically.

Push **START** key

“Run” mode (the protocol name in IC card is displayed, and then initialized the instrument automatically)

Sample information screen appears. Select the type of sample.

Select DNase treatment.

RNA PURIFICATION
--CELL/TISSUE--
-- PSSJ Ver.1.0/ 6GC--
Initializing...

SELECT SAMPLE
1. CELL
2. TISSUE
NEXT=1or2, ESC=Prev.

DNase TREATMENT
1. YES
2. NO
NEXT=1or2, ESC=Prev.

START

Select the elution volume.

SELECT ELUTION VOL.
1. 50µl
2. 100µl
NEXT=1or2, ESC=Prev.

Display example

SAMPLE: TISSUE
DNase : YES
ELUTION: 50µl
NEXT=RETURN, ESC=Prev.

Push key

Push **START** key

Run

* It “beeps” after completion of running.

Do Not insert or remove the IC card while the power is on.

This may result in damage to the system.

Do Not open the front door during operation because the system stops suddenly.



Extraction Process - RNA Purification -

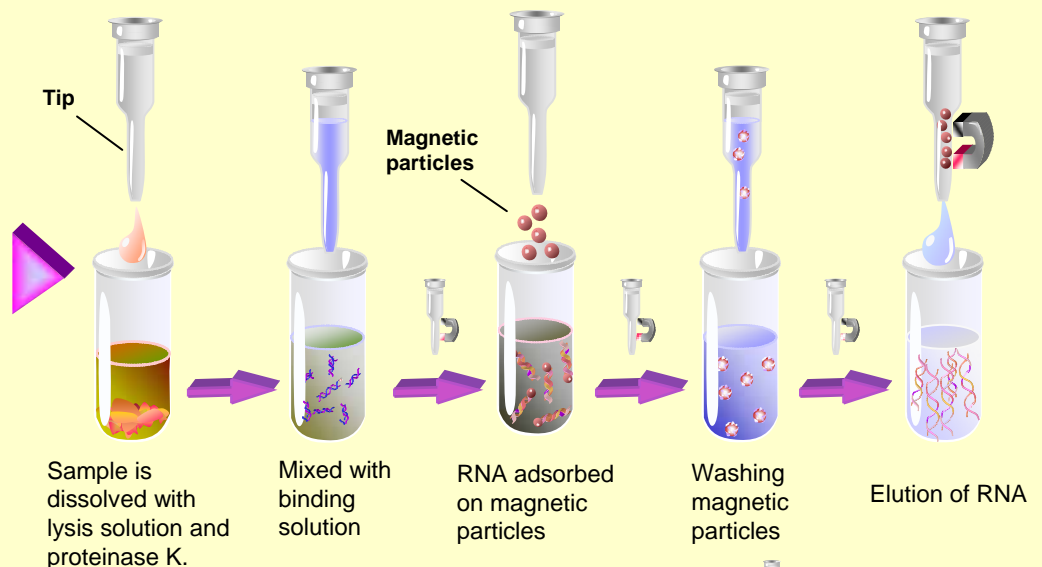
Tissue
+ RNAlater®
150 µl
Crushed tissue solution 150 µl

Homogenize

Cell
+ Physiological saline 200µl
Cell suspension 200µl

Pipetting or Vortex

Sample pretreatment



: Magnetic separation of magnetic particles



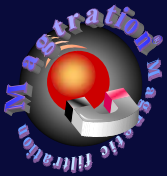
Troubleshooting

Troubles

Possible causes

Comments

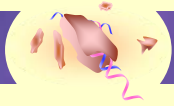
(1) Low yield		Excessive sample amount	If a sample amount larger than the prescribed amount is used, the yield will decrease rather than increase. Try to reduce the amount of the sample. The optimal amount is 1×10^6 cells or less per 200 μ l, or 10 mg or less per 150 μ l for tissue samples.
		Insufficient sample lysis	This can result from insufficient stirring or crushing of the sample. If the sample loaded on the system contains solid materials due to insufficient crushing, tips may become clogged during the extraction procedure, resulting in poor yields.
		Insufficient sample crushing	This can result from insufficient stirring or crushing of the sample. If the sample loaded on the system contains solid materials due to the insufficient crushing, the sample may clog the tip or become less lysating in the process. These can cause poor yields. Tissue crushing should be performed to such a degree, easy pipetting can be achieved with a 1000 μ l pipette.
		Tip clogging	Samples should be crushed completely, since some tissue may contain a large proportion of muscle fibers. Refer to the comment above.
		Condition of the pre-filled reagent	In the case of air bubble formation in the cartridge reagent, adhesion of reagent, or formation of droplets in the sealed part of the reagent or on the upper part of the well, tap the cartridge or the well lightly to remove them. Processing without removing the air bubbles will lead to incomplete aspiration of the reagent or generate more bubbles at the stirring step, resulting in reduced yield. If the magnetic particles are attached to the wall of the well, it is important to remove them by tilting the cartridge with gentle agitation. Ensure that no bubbles are formatted.
(2) Low A260/A280		Excessive sample amount	Try to reduce the amount of the sample. Refer to (1).
		RNA concentration	If RNA concentration is low, A260/A280 ratio may become low.
		Contamination by magnetic particles	The contamination by magnetic particles may result in background (A320) elevation or noise. For determination of the absorbance or an assay using Agilent 2100 Bio Analyzer, centrifugation of the supernatant for 1 min (10,000g) is recommended.
(3) Degradation of RNA		Excessive sample amount	When excessive amount of sample are used, RNase may not be inactivated sufficiently. Try to reduce the amount of sample used.
		Eluted solution left unattended	Do not leave the eluted sample at room temperature after processing. After completion of extraction, immediately seal the elution tube and store it at -80°C .
		Effect of freezing and thawing	Freezing and thawing of samples after extraction may affect the quality of the RNA. Care should be taken especially when the RNA level of the sample is low. We recommend analysis of the samples immediately after extraction.
		Selection of DNase	Degradation of the RNA may occur due to DNase I treatment. Try to use RNase-free DNase I or the DNase I recommended by the manufacturer. For DNase buffer, it is also advisable to use an RNase-free product.
(4) RT-PCR failure		Contamination by genomic DNA	If a pseudo-gene possessing an identical sequence to the mRNA exists in the genome, it is necessary to consider its potential influence in PCR. If possible, use a protocol containing DNase treatment with 6GC/12GC.
		Selection of DNase	Refer to (3).
		Excessive sample amount	Try to reduce the amount of the sample. Refer to (1), (2), and (3).
(5) Coloration of eluted solution		Contamination by hemoglobin or chlorophyll	When using the sample containing a large amount of hemoglobin or chlorophyll, the eluent may be colored. In this case, reduce the amount of the sample, or avoid contamination by blood components as much as possible, especially for tissue samples.
		Contamination by magnetic particles	The eluate may appear darkly colored due to contamination by magnetic particles. Though this does not affect enzymatic reactions, the particles can be removed by centrifugation. Refer to (2).
(6) Agglutination of magnetic particles		Excessive sample amount	Try to reduce the amount of the sample. Refer to (1), (2), and (3).



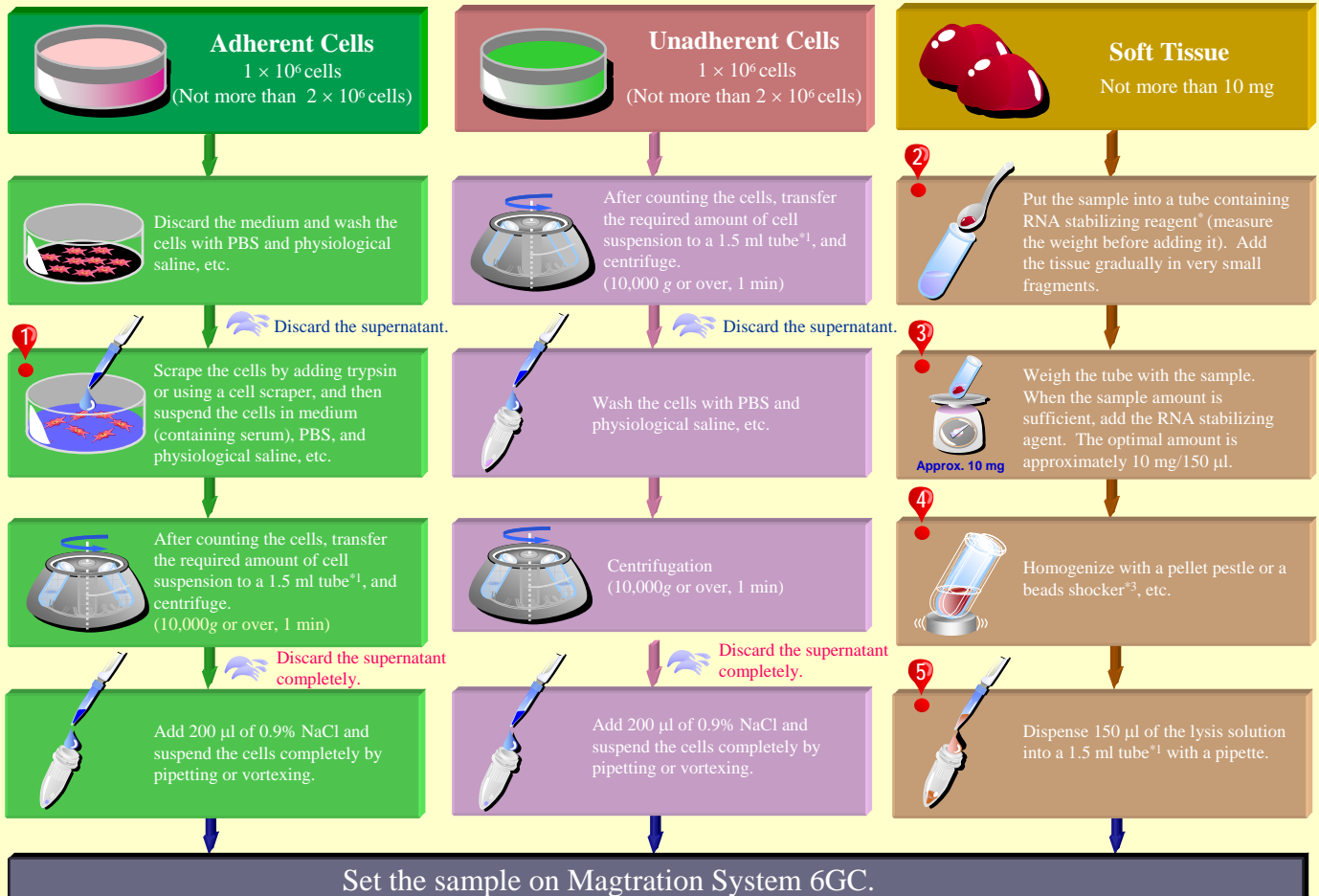
Magratron System 6GC

Automated Nucleic Acid Isolation and Purification System

Quick Protocol ~ RNA Purification ~



Sample Preparation

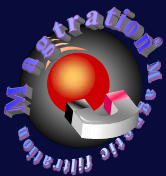


- 1 Add PBS containing 0.1-0.25% trypsin to the dish and scrape the cells. Add culture medium containing serum after completion of scraping, and then centrifuge. Wash the cells at least once after centrifugation.
- 2 After powderizing the tissue sample with pestle and mortar under liquid nitrogen, add the tissue sample into the RNA stabilizing agent. It may be difficult to absorb the total amount of lysate solution using some types of beads used for the beads shocker. In such cases, add larger quantities of RNA stabilizing agent.
- 3 The use of a large quantities of tissue samples will not cause any processing problems, but may decrease the stability of the RNA. The optimal amount is approximately 10 mg/150 µL.
- 4 Tissue crushing should be performed to such a degree that easy mixing can be achieved with a 1000 µL pipette, without blocking the tip.
- 5 Dispense the following amount into a 1.5 ml screw cap tube:
 - 150 µL tissue lysis solution, or
 - 200 µL cell suspension.

- *1 Recommended tube: Assist
: 1.5 ml frozen tube (Cat# 72.692S or 72.687S)
- *2 Recommended reagent: Ambion: RNAlater[®] (Cat# 7020)
- *3 Recommended crusher:
QIAGEN: Tissue Lyser (Cat# 85200)
Roche Diagnostics: MagNA Lyser (Cat# 3358968)

Refer to the operating manual or handbook distributed by the above manufacturers for details regarding the processing conditions of various tissues.

Please note that sample amounts greater than the amounts prescribed cannot be aspirated on the Magratron System.

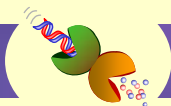


Magratration System 6GC

Automated Nucleic Acid Isolation and Purification System

Quick Protocol

~ RNA Purification ~



Preparation of DNase I



Recommended to DNase

- DNase I RNase-free (Ambion , Cat. No. 2222)
- DNase I, Amplification grade (Invitrogen, Cat. No. 18068-015)
- DNase I (RNase-free) (TaKaRa, Cat. No. 2215A)
- RQ1 RNase-Free DNase (Promega, Cat. No. M6101)
- Deoxyribonuclease (RT Grade) (Nippon Gene, Cat. No. 313-03161)
- DNase I, RNase-free (Roche, Cat. No. 776785)



Recommended to x10 DNase buffer

- DNase I buffer x10 (Ambion , Cat. No. 8170G)
- x10 DNase (RT Grade) buffer (Nippon Gene, Cat. No. special-order item)



Preparation of DNase

1. Use 1.5ml tube supplied in the Magratration®-MagaZorb® RNA common kit.
2. Prepare recommended DNase I and x10 DNase buffer.
3. Make DNase solution, refer to the table below.



Follow the preparation table below. Prepare the DNase I solution in total 80-100µl in a tube. Ensure that the combination of each DNase I and DNase buffer is required the different volumes in the solution. After making the DNase solution, set the tube into the right position in Magratration® System 6GC/12GC.

Example : In case of using DNase(TaKaRa) and DNase buffer(Nippon Gene).
 ⇒Add 8µl DNase I, 30µl x10 buffer, and 60µl x10 BSA and mix then in a 1.5ml tube to make 98µl DNase solution.

The quantity of DNase I

DNase I	DNaseI (µl)
Ambion, DNaseI RNase-free	20 [2U/µl]
Invitrogen, DNaseI, Amplification grade	1 [114U/µl]
TaKaRa, DNaseI (RNase-free)	8 [5U/µl]
Promega, RQ1 RNase-Free DNase	40 [1U/µl]
NipponGene, Deoxyribonuclease (RT Grade)	40 [1U/µl]
Roche, DNaseI, RNase-free	4 [10U/µl]



The quantity of DNase Buffer

A Appending buffer (µl)	B Ambion x10 buffer (µl)	C NipponGene x10 (RT-Grade)buffer	
		x10 buffer (µl)	x10 BSA (µl)
⇒ B	80	30	50
	80	30	60
80	80	30	60
60	60	40	20
⇒ C	60	40	20
	80	30	60