

Magnetic Hi-Tissue/Cell Total RNA Kit

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Magnetic Hi-Tissue/Cell Total RNA Kit

Cat. no. GDP771

Kit Contents

Product composition		GDP771 (96 preps)
GDP771H	RNAstore Reagent	12 ml
	Buffer TSA	70 ml
	Proteinase K	2 × 1 ml
	Buffer ICBP	35 ml
	MagAttract Suspension BE (100 mg/ml)	2 × 1 ml
	Buffer RDC	90 ml
	Buffer RW1A	200 ml
	Buffer RW4	90 ml
	Buffer RW	20 ml
	RNase-Free ddH ₂ O	30 ml
GRT431	RNase-Free DNase I (1500 U)	1 pcs
	RNase-Free ddH ₂ O	1 ml

Note: GRT431, GDP771H are shipped and stored separately.

Storage

All components of the kit GDP771H can be stored in dry conditions at room temperature (15-30°C) for 15 months. RNase-Free DNase I and RNase-Free ddH₂O can be stored at 2-8°C for 15 months.

Introduction

The kit adopts magnetic beads and a unique buffer system, to isolate and purify total RNA with high quality from cell/tissue. The uniquely embedded magnetic beads have a strong affinity for nucleic acid under certain conditions. When the conditions are changed, the magnetic beads can release the absorbed nucleic acid to rapidly separate and purify it.

It can be used to perfectly fit with automated nucleic acid extractor. Through absorption, transfer and release of magnetic beads by the special magnetic bar, magnetic beads and nucleic acid can be transferred to improve the degree of automation. The whole process is safe and convenient, and the extracted RNA has high purity. If high throughput automated extraction is required, TIANGEN can deliver integrated solutions.

RNA purified by this kit is suitable for downstream experiments including RT-PCR, Real Time RT-PCR, chip analysis, Northern Blot, Dot Blot, PolyA screening, in vitro translation, RNase protection analysis and molecular cloning.

Product Features

Simple and fast: Ultra-pure total RNA can be obtained quickly.

Safe and non-toxic: No toxic reagents such as phenol/chloroform.

High purity: The RNA obtained has high purity and can be directly used for chip detection, high-throughput sequencing and other experiments.

Notes Please be sure to read this precaution before using the kit.

1. Replace the gloves with new ones frequently. This is because skin often carries bacteria that can lead to RNase contamination.
2. Avoid cross-contamination by using RNase-free plastics and tips.
3. Pay attention to optimal sample storage and pretreatment conditions to avoid causing degradation of the extracted RNA.

Reagents need to be prepared by Customer

Isopropanol (supplementary protocol: samples preserved in Trizol), anhydrous ethanol, homogenization equipment (mortar, homogenizer, etc.), liquid nitrogen, gloves, masks, RNase-Free centrifuge tubes, magnetic racks, or nucleic acid extractor, for other brands of extractor to be integrated, please contact TIANGEN.

Recommended dosage for different types of samples

Note: Please refer to the recommended ranges in the table below. Do not exceed 100 mg of tissue, otherwise it may lead to RNA yield and quality decrease.

	Sample type	Optimal extraction amount
Tissue	Spleen	5 mg
	Liver	10 mg
	Kidney	15 mg
	Intestine	15 mg
	Gland	15 mg
	Heart	25 mg
	Lung	25 mg
	Brain	25 mg
	Fat	50 mg
	Skin	100 mg
	Muscle	100 mg
	Tail	0.3 cm for rats, 0.6 cm for mice
Cell	Non-stop protocol	10^5 - 10^7 cells
	Low yield, pause protocol recommended	Less than 10^5 cells
Insects	Mollusc	25 mg
	Crustacea	50 mg
Fungus	Yeast	Not more than 5×10^7
	Mould	25 mg
	Mushrooms	75 mg

Preparation of DNase I storage solution

Dissolve DNase I dry powder (1500 U) in 1 ml RNase-Free ddH₂O, mix gently and follow the protocol to add 10 µl to each sample for freshly use.

Attention: If long-term storage needed, dissolve DNase I dry powder (1500 U) in 550 µl RNase-Free ddH₂O to make stock solution, mix gently, dispense and store at -30~-15°C (can be stored for 9 months). Add 5 µl stock solution to each sample. Store the DNase I stock solution after thawing from -30~-15°C at 2-8°C (can be stored for 6 weeks) and do not freeze it again.

Protocol

A. Manual operation procedure

Add 100% ethanol to the Buffer RW before use, and refer to the label on the bottle for the volume to be added.

I Sample pre-treatment

1. Tissue sample

1) Sample pre-treatment

- Grinding by liquid nitrogen. Take 5-100 mg tissue in liquid nitrogen and quickly grind it into powder, add 100 μ l RNA Store, 600 μ l Buffer TSA and 20 μ l Proteinase K, immediately vortex and mix, let it stand at room temperature for 5 min.
- Grinding by Homogenizer. Take 5-100 mg tissue, add 100 μ l RNA Store and grinding beads(not provide, TIANGEN, Cat.no OSE-TH-B03). Homogenize with TGrinder H24(not provide, TIANGEN, Cat.no OSE-TH-02) and homogenize with a program of 30 sec of oscillation at 6.5 m/s for a total of 1 cycle. Add 600 μ l Buffer TSA and 20 μ l Proteinase K, vortex and mix, let it stand at room temperature for 5 min.

Note: Pre-mixing can be done in the ratio of 100 μ l RNAstore, 600 μ l Buffer TSA, and 20 μ l Proteinase K. Add 720 μ l per sample, mixing it freshly.

- Centrifuge it at 12,000 rpm ($\sim 13,400\times g$) for 5 min, and carefully take 600 μ l supernatant for the subsequent experiments.
- Slowly add 200 μ l Buffer ICBP, mix thoroughly, add 20 μ l of MagAttract Suspension BE (100 mg/ml).

Note 1 Vortex of MagAttract Suspension BE (100 mg/ml) well before use.

Note 2 Enhance to 300 μ l ICBP if sample input as micro level.

Note 3 Supplementary protocol: For tissue sample preserved in Trizol, after homogenization, centrifugation at 8,000 rpm ($\sim 7104\times g$) for 2 min, vortex thoroughly and take 600 μ l supernatant. Adding 250 μ l isopropanol and 20 μ l of MagAttract Suspension BE (100 mg/ml) to the supernatant and continuing the subsequent process.

2. Cell sample

1) Cell Collection

- For suspension cells (please do not collect more than 1×10^7 cells): Estimate the number of cells, centrifuge at $300\times g$ for 5 min, collect the cells into a centrifuge tube, and carefully discard all the culture medium.
- Trypsin treatment method: determine the number of cells, no more than 1×10^7 , discard the medium, wash the cells with PBS solution. Discard the PBS solution, and add PBS solution containing 0.10-0.25 % trypsin to detach the cell from the wall. Add medium containing serum to inactivate the trypsin then transfer the cell solution to RNase-Free centrifugation tubes. Centrifuge at $300\times g$ for 5 min, carefully discard all supernatant and collect the cell pellets.

Note 1: Be sure to remove the cell culture when collecting the cells, otherwise it will lead to incomplete lysis and will affect the binding of RNA to the magnetic beads, which resulting in a lower yield of RNA.

Note 2:PBMC separated from whole blood can be with above protocol.

- 2) Add 100 µl of RNA Store, 600 µl of Buffer TSA and 20 µl of Proteinase K to the cell pellets, vortex immediately, let it stand at room temperature for 5 min, and carefully take 600 µl of supernatant for subsequent experiments.
- 3) Slowly add 200 µl Buffer ICBP (enhance to 300 µl if cell number less than 10^5), mix thoroughly, add 20 µl of MagAttract Suspension BE (100 mg/ml).

Note Supplementary protocol: For cell pellet preserved in Trizol, after homogenization or vortex, take 600 µl sample then add 250 µl isopropanol and 20 µl MagAttract Suspension BE (100 mg/ml) and continuing the subsequent.

3. Blood sample

1) Rapid protocol for direct lysis of fresh blood

- a. Take 150 µl of fresh anticoagulant blood, add 450 µl of Buffer TSA and 20 µl of proteinase K, immediately vortex and mix, and let it stand at room temperature for 5 min, carefully aspirate 600 µl supernatant for subsequent experiments.
- b. Slowly add 300 µl Buffer ICBP, mix thoroughly, and add 20 µl of MagAttract suspension BE (100 mg/ml).

2) Fresh blood pretreatment program

- a. Take no more than 1 ml of fresh anticoagulated blood solution and add RNALock Reagent at the ratio of 1:5 (not provided, TIANGEN, Cat.no GDP440), e.g., take 300 µl of fresh anticoagulant blood and add 1.5 ml of RNALock Reagent.

Note: Make sure RNALock Reagent is stored at room temperature before use.

- b. Immediately cap the tube and mix 8-10 times upside down.

Note: If storage is required, please refer to the storage conditions of RNALock Reagent (customer supplied, TIANGEN, Cat. no GDP440-02) instructions

- c. Then centrifuge at 6,500 rpm ($\sim 4,000\times g$) for 10 min, pipette off the supernatant, and remove the precipitate as follows.

Note: If clumping or insufficient cleavage is evident after centrifugation, repeat steps a-c.

- d. Add 1 ml of RNase-Free ddH₂O (supplied by customer) to the precipitate and pipette to completely dissolve the precipitate.
- e. Then centrifuge at 6,500 rpm ($\sim 4,000\times g$) for 10 min and discard the supernatant by pipetting.

- f. Slowly add 150 μ l of Suspension RSB (customer supplied, TIANGEN, content of Cat. no GDP440) and pipette repeatedly to dissolve the precipitate.
- g. Add 450 μ l Buffer TSA and 20 μ l of proteinase K, vortex and mix immediately, let it stand for 5 min at room temperature, and then carefully aspirate 600 μ l of supernatant for subsequent analysis.
- h. Slowly add 300 μ l Buffer ICBP, mix well, add 20 μ l of MagAttract suspension BE (100 mg/ml).

3) Blood storage in RNAlock or PAXgene tube

- a. To purify blood samples preserved in RNAlock and PAXgene tube, first bring the samples to bench or a 37°C water bath to balance them to room temperature. Take appropriate volume of sample and centrifuge at 6,500 rpm ($\sim 4,000\times g$) for 10 min (The maximum extraction capacity is 1 ml whole blood for each prep). Pipette off the supernatant and take the precipitate for the following operation.
- b. Add 1 ml of RNase-Free ddH₂O (supplied by customer) to the precipitate and pipette to completely dissolve the precipitate.
- c. Then centrifuge at 6,500 rpm ($\sim 4,000\times g$) for 10 min and pipette off the supernatant.
- d. Slowly add 150 μ l of Buffer RSB (customer supplied, TIANGEN, content of Cat. no GDP440) and pipette to dissolve the precipitate completely.
Note: 150 μ l Buffer RSB can be replaced by 150 μ l buffer PBS if using PAXgene tube.
- e. Add 450 μ l of Buffer TSA and 20 μ l of proteinase K, immediately vortex and mix, let stand at room temperature for 5 min, and then carefully aspirate 600 μ l of supernatant for subsequent processing.
- f. Slowly add 300 μ l Buffer ICBP, mix thoroughly, and add 20 μ l of MagAttract suspension BE (100 mg/ml).

4. Bacteria, fungi, environmental, processed food samples

1) Pre-processing of samples

- a. Cultured bacteria (for both Gram positive and Gram negative bacteria)

Centrifuge at 12,000 rpm ($\sim 13,400\times g$) for 2 min to collect bacteria (the maximum amount of bacteria collected should not exceed 1×10^9), carefully remove all culture medium supernatant, and all subsequent steps are performed at room temperature.

Note: Incomplete removal of the medium will inhibit the cell wall digestion process.

- b. Environmental Samples

Silt and sediment samples: take 50-100 mg of silt and sediment samples and add them to the centrifuge tube.

Water samples: Take a certain volume of water and filter it through a membrane, then cut up the membrane and put it into a centrifuge tube (or, depending on the sample, centrifuge it at high speed and add 50-100 mg of precipitate to the centrifuge tube for the next step).

c. Food samples

Yogurt samples: Take 1-2 ml of sample and centrifuge at 6,500 rpm (~4,000×g) at 4°C for 2 min to collect the precipitate.

Soy sauce: take 10-50 ml of sample, centrifuge at 6,500 rpm (~4,000×g) for 2 min at 4°C to collect the precipitate.

Wine fermentation samples: take 1-5 ml of fermentation intermediates, centrifuge at 6,500 rpm (~4,000×g) for 2 min at 4°C to collect the precipitate. Solid samples can be taken as ~100-200 mg samples for extraction.

Note 1: Since the microbial content of deep-processed samples varies greatly at different stages of processing, a suitable sample volume can be taken according to the actual sample conditions.

Note 2: For samples with more impurities, add 1 ml of water after centrifuge 2 min at 6,500 rpm (~4,000×g) to collect the precipitate and wash it again.

d. Yeast

Take 1-2 ml of yeast cells (maximum 5×10^7 cells) and centrifuge at 12,000 rpm (~13,400×g) for 1 min, and aspirate the supernatant as much as possible (when there is a large amount of bacterial fluid, the bacterial precipitate can be collected by several centrifugations into a centrifuge tube).

e. Molds

Mycobacteria cultured in liquid medium were collected by centrifugation at 12,000 rpm (~13,400×g) for 2 min and take 30-50 mg of mycobacteria for the next step. When culturing with solid medium, scrape mycelium from the surface and take 30-50 mg of mycobacteria for the next step in the experiment.

Note: Mycobacterial samples can also be ground in liquid nitrogen, omitting the enzyme digestion steps in steps 2) and 3) and proceeding directly to step 4) Add lysate for subsequent steps.

- 2) Thoroughly resuspend the bacteria with 400 µl of Buffer LY (not provided, TIANGEN, Cat.no GRT401-11), add 50 µl of Lysozyme A (not provided, 50 mg/ml, TIANGEN, Cat.no GRT401-11) and 2 µl of Lyticase A (not provided, 10 U/µl, TIANGEN, Cat.no GRT410-12), incubate at 37°C for 15 min.

Note: If only focusing on bacteria, you can add only Lysozyme A for digestion; If focusing on fungi only, you can add only Lyticase A for digestion.

- 3) Centrifuge at 6,500 rpm (~4,000×g) for 2 min and discard supernatant.

- 4) Add 650 μ l of Buffer TSA and 20 μ l of Proteinase K. Suspend the precipitate and transfer it to a homogenization tube (provided by the customer, TIANGEN, Cat. no OSE-TH-B06) and mix for 15 min with shaking at 1,200 rpm. Or TGrinder H24R Tissue Homogenizer (provided by the customer, TIANGEN, Cat.no OSE-TH-02) (temperature -10°C, 6M/S speed oscillation for 30 sec, 1 cycle).
- 5) After homogenization, centrifuge at 12,000 rpm ($\sim$ 13,400 $\times$ g) for 2 min, and carefully aspirate 600 μ l of supernatant for subsequent tests.
- 6) Slowly add 300 μ l of Buffer ICBP, mix thoroughly, and add 20 μ l of MagAttract suspension BE (100 mg/ml).

5. Stool sample

- 1) Weigh 50-100 mg of fecal sample, use 400 μ l of Buffer LY (not provided, TIANGEN, Cat.no GRT401-11) to mix thoroughly. The sample should be thoroughly resuspended and dispersed as much as possible without obvious lumps.

Add 50 μ l of lysozyme A (not provided, 50 mg/ml, TIANGEN, Cat.no GRT401-11) and 2 μ l of lyticase A (not provided, 10 U/ μ l, TIANGEN, Cat.no GRT410-12) and incubate at 37°C for 15 min.

Note1: If only focusing on bacteria, you can add only Lysozyme A for digestion; If focusing on fungi only, you can add only Lyticase A for digestion.

Note2: For samples with more impurities, add 1 ml of water after centrifuge 2 min at 6,500 rpm ($\sim$ 4,000 $\times$ g) to collect the precipitate and wash it again then carry out the enzymatic digestion.

- 2) Centrifuge at 6,500 rpm ($\sim$ 4,000 $\times$ g) for 2 min and discard the supernatant.
- 3) Add 650 μ l of Buffer TSA and 20 μ l of Proteinase K. Suspend the precipitate and transfer to a homogenization tube (provided by the customer, TIANGEN, Cat.no OSE-TH-B06) and mix for 15 min with shaking at 1,200 rpm. Or TGrinder H24R Tissue Homogenizer (provided by the customer, TIANGEN, Cat.no OSE-TH-02) (temperature -10°C, 6M/S speed oscillation for 30 sec, 1 cycle).
- 4) After homogenization, centrifuge at 12,000 rpm ($\sim$ 13,400 $\times$ g) for 2 min, and carefully aspirate 600 μ l of supernatant for the subsequent experiments.
- 5) Slowly add 300 μ l of Buffer ICBP, mix thoroughly, and add 20 μ l MagAttract suspension BE (100 mg/ml).

II Purification and elution

1. Follow the above process to carry out the appropriate sample pre-treatment and enzymatic digestion and other processes.
2. Shake and mix for 5 min, place the centrifuge tube on a magnetic rack for 30 sec-1 min, and carefully remove the liquid when the magnetic beads are fully attached.

3. Add 900 μ l of Buffer RW1A, shake and mix for 1-3 min, place on a magnetic rack for 30 sec-1 min, and remove the supernatant.
4. Add 700 μ l Buffer RDC solution and 10 μ l DNase I working solution, mix gently, place at room temperature for 15 min, place on a magnetic rack for 30 sec-1 min for static adsorption, remove supernatant.
5. Add 700 μ l of Buffer RW1A, shake and mix for 1-3 min, place on a magnetic rack for 30 sec-1 min, and remove the supernatant.
6. Add 700 μ l RW4, shake and mix for 1-3 min, place on a magnetic rack for 30 sec-1 min, and remove supernatant.
7. Add 700 μ l Buffer RW (please check whether 100% ethanol has been added before use), shake and mix for 1-3 min, place on a magnetic rack for 30 sec-1 min, and remove supernatant.
8. Dry at room temperature for 3-5 min.

Note: Ethanol residue will inhibit subsequent enzymatic reactions, so make sure the ethanol evaporates cleanly when drying. However, do not dry for too long as it may be difficult to elute the RNA.

9. Add 50-100 μ l RNase-Free ddH₂O, mix gently, leave it at room temperature for 5 min (heating elution at 45°C can improve the yield), place it on a magnetic rack for 2 min, and transfer the supernatant into a new EP tube.

Note: RNA samples should be stored at -90~-65°C.

B. TGuide S96 Nucleic Acid Extractor automated process

Add 100% ethanol to the Buffer RW before use, and refer to the label on the bottle for the volume to be added.

I. Sample pre-treatment

1. Buffer ICBP and MagAttract Suspension BE and dispense according to step II.
2. Refer to the manual procedure for the pretreatment of different samples until the end of "Carefully aspirate 600 μ l of supernatant for subsequent experiments".

II. Dispense the solution according to the following position

Plate	E	F	G	H
Reagent	Buffer RW1A 900 µl	DNase I 10 µl RDC 700 µl	Buffer RW 700 µl MagAttract Suspension BE 20 µl Tip comb	
Plate	A	B	C	D
Reagent	Sample supernatant 600 µl Buffer ICBP 200-300 µl	Buffer RW1A 900 µl	Buffer RW4 800 µl	RNase-Free ddH ₂ O 100 µl

Note 1 Use fresh DNase I and Buffer RDC to avoid decrease DNase I activity.

Note 2 Enhance to 300 µl ICBP if cell number less than 10⁵.

Note 3 Supplementary protocol: For tissue sample preserved in Trizol, after homogenization, centrifugation at 8,000 rpm (~7104×g) for 2 min, vortex thoroughly and transfer 600 µl supernatant to plate A. Adding 250 µl isopropanol instead of Buffer ICBP to plate A and continuing the subsequent process.

Note 4 Supplementary protocol: For cell pellet preserved in Trizol, after homogenization or vortex, transfer 600 µl sample to plate A. Adding 250 µl isopropanol instead of Buffer ICBP to plate A and continuing the subsequent process.

III. TGuide S96 Nucleic Acid Extractor

1. Insert the Tip Combs into the MagAttract Suspension BE beads plate. Run the TGuide S96 Automatic Nucleic Acid Extraction program below :

Steps	Plate position setting	Mixing volume (ul)	Mix speed	Mix time (min)	Sedimentation time (sec)	Number of magnetic aspirations	Magnet speed (mm/s)	Heating plate location	Heating temperature (°C)	Suspension time (min)	Automatic pause	Gripper action
Tip	G	—	—	—	—	—	—	—	—	—	—	Gripping
Collect Beads	G	800	Mid/slow speed	0.5	10	1	0.8	—	—	—	—	—
Mixing	E	900	Mid/slow speed	0.5	—	—	—	—	—	—	—	—
Mixing	A	800	Mid/slow speed	2	—	—	—	—	—	—	—	—
Collect Beads	E	900	Mid/slow speed	0.2	10	1	0.8	—	—	—	—	—
Binding	A	800	Mid/slow speed	5	10	2	0.8	—	—	—	—	—
Wash-I	E	900	Mid	5	10	1	1	—	—	—	—	—
DNaseI	F	710	Mid	12	10	1	0.8	—	—	—	—	—
Wash-II	B	900	Mid	5	10	1	1	—	—	—	—	—
Wash-III	C	800	Mid	4	10	1	1	—	—	—	—	—
Wash-IV	G	720	Mid	4	10	2	1	—	—	5	—	—
Elution	D	100	Mid/slow speed	6	10	3	0.8	—	45	—	—	—
Finish	C	—	—	—	—	—	—	—	—	—	—	Release

2. At the end of the extraction experimental procedure of the TGuide S96, the RNA in the 96 deep-well plate at the D plate position is aspirated and stored under -90~-65°C.

C. TGuide S16 Nucleic Acid Extractor automated process

Add 100% ethanol to the Buffer RW before use, and refer to the label on the bottle for the volume to be added.

I. Sample pre-treatment

1. Buffer ICBP and MagAttract Suspension BE and dispense according to step II.
2. Refer to the manual procedure for the pretreatment of different samples until the end of "Carefully aspirate 600 μ l of supernatant for subsequent experiments".

II. Dispense the solution according to the following position

Column 1/7	Column 2/8	Column 3/9	Column 4/10	Column 5/11	Column 6/12
ICBP	RW1A	DNase I RDC	RW4	ddH ₂ O	RW MagAttract Suspension BE
Sample supernatant 200-300 μ l	900 μ l	10 μ l 700 μ l	800 μ l	100 μ l	700 μ l 20 μ l

Note 1 To avoid affecting DNase I activity, add DNase I to Column 3/9 just before run.

Note 2 Enhance to 300 μ l ICBP if cell number less than 10^5 .

Note 3 Supplementary protocol: For tissue sample preserved in Trizol, after homogenization, centrifugation at 8,000 rpm ($\sim 7104\times g$) for 2 min, vortex thoroughly and transfer 600 μ l supernatant to Column 1/7. Adding 250 μ l isopropanol instead of ICBP to Column 1/7 and continuing the subsequent process.

Note 4 Supplementary protocol: For cell pellet preserved in Trizol, transfer 600 μ l sample to Column 1/7 after homogenization or vortex. Adding 250 μ l isopropanol instead of ICBP to Column 1/7 and continuing the subsequent.

III. TGuide S16 Nucleic Acid Extractor.

1. Insert the Tip Combs into the slots to ensure that they are well connected and firmed. Run the TGuide S16 Automatic Nucleic Acid Extraction program below:

Step	Hole site	Name	Mix time (min)	Mix speed	Dry time (min)	Volume (μl)	Temp. (°C)	Segments	Every time (s)	Magnetization time (s)	Cycle	Magnet speed (mm/s)
1	6	Pipette beads	0.5	7	0	720	--	5	5	3	2	2
2	2	Collect beads	0.5	7	0	900	--	1	0	0	0	--
3	1	Lysis	2	8	0	800	--	1	0	0	0	--
4	2	Pipette beads	0.5	7	0	900	--	5	5	3	2	2
5	1	Bonding	5	8	0	800	--	5	5	3	2	2
6	2	Wash 1	3	7	0	900	--	5	5	0	2	2
7	3	DNase I	12	3	0	710	--	1	5	0	2	2
8	2	Wash 2	5	7	0	900	--	5	5	3	2	2
9	4	Wash 3	3	7	0	800	--	5	5	0	2	2
10	6	Wash 4	3	7	6	720	--	5	5	0	2	2
11	5	Elution	5	7	0	100	45	5	5	5	2	2
12	6	Discard beads	0.5	5	0	720	--	1	0	0	0	--

2. At the end of the automated extraction process, take the RNA out of the Column 5/11 of the 96-Deepwell plate and store it under -95~-65°C.

RNA purity and concentration test

Integrity: RNA can be detected by agarose gel electrophoresis (gel concentration 1.2%; 0.5×TBE buffer; 150 V for 15 min) to detect the integrity. Since 70-80% of the RNA in the cell is rRNA, it should be visible under UV after electrophoresis. The size of rRNA is about 5 kb and 2 kb, equivalent to 28S and 18S rRNA, respectively.

RNA integrity can also be quantitatively assessed by capillary electrophoresis (e.g. Agilent 2100 Bioanalyzer or 4200 TapeStation system), and the core metric is RNA Integrity Number (RIN). For routine samples, the RIN value ranges from 1-10, with higher values representing lower RNA degradation; for special samples, such as formalin-fixed paraffin-embedded (FFPE) samples, the fixation process may lead to

severe RNA degradation, and the RIN value may not accurately reflect the degree of degradation. It is recommended to combine with the DV200 index (i.e., the proportion of RNA fragments >200 nt) for comprehensive evaluation.

Purity: The OD_{260}/OD_{280} ratio is a measure of protein contamination. For high quality RNA, OD_{260}/OD_{280} readings are in the range of 1.8-2.0. The OD_{260}/OD_{280} ratio is a measure of protein contamination, and for high-quality RNA, the OD_{260}/OD_{280} reading is between 1.8 and 2.1, with a ratio of 2.0 being an indicator of high-quality RNA. OD_{260}/OD_{280} readings are affected by the pH of the solution used for the assay. For the same RNA sample, it is assumed that OD_{260}/OD_{280} readings in 10 mM Tris, pH 7.5, range from 1.8-2.1, while those in aqueous solutions range from 1.8-2.1. OD_{260}/OD_{280} readings of 1.8-2.1 in a 10 mM Tris, pH 7.5 solution are assumed to be in the range of 1.8-2.1, whereas readings in an aqueous solution may be in the range of 1.5-1.9, which is not indicative of impure RNA.

Concentration: Take a certain amount of RNA extract, dilute n-fold with RNase-Free ddH₂O, and zero the spectrophotometer with RNase-Free ddH₂O. Zero the spectrophotometer, take the diluted solution for OD_{260} , OD_{280} measurement, and carry out the calculation of RNA concentration according to the following formula:

Final concentration (ng/μl) = (OD_{260}) × (dilution times) × 40