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Version 1.0
September 2021

GeneMAGNET RNA Purification Kit

Kit for isolation of total RNA from tissues, bacteria, yeast, cell culture and blood (leukocytes).

Cat. no. E3401

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Table of Contents

Kit content	96 preps E3401-01	Storage/Stability
RL	44 ml	15-25°C
Wash RBW	64 ml	15-25°C
Wash RBW2	32 ml	15-25°C
DNR	11 ml	15-25°C
RNase-free water	12 ml	15-25°C
Magnetic Beads	2000 µl	2-8°C
DNase I (5U / 1 µl)	4 x 275 U	15-25°C
DNase I buffer	300 µl	2-8°C
Protocol	1	

Introductory Notes

NOTE 1 · Kit Specification. This universal kit is designed for isolation of total RNA from animal tissues, cell culture, yeast, any Gram+ and Gram- bacteria and from blood (leukocytes).

NOTE 2 · Maximum Magnetic Beads Binding Capacity. The total RNA binding capacity is 80 µg per 10 µl of beads.

NOTE 3 · DNase I Digestion. The procedure employs DNA digestion by DNase I that is included in the kit. DNase I should be dissolved in DNase I buffer prior to starting the RNA purification.

NOTE 4 · Kit Compounds Storage. Keep all solutions tightly closed to avoid evaporation, resulting in components concentration changes. After shiping store Magnetic Beads and DNase I buffer in 2-8°C and all other components of the kit at room temperature. Store DNase I after dissolving in DNase I buffer in -20°C.

NOTE 5 · Maintaining Good Working Practice. To obtain high quality RNA, stick carefully to the protocol provided below. One of the most critical issues during RNA isolation is, to ensure working quickly and with practiced hand. RNA isolation should be performed at room temperature throughout the entire process. Avoid introducing any RNases during the procedure or later handling.

NOTE 6 · β-Mercaptoethanol / DTT. Contaminating RNases are inactivated by addition of reducing agents capable of disrupting disulfide bonds, such as ß-mercaptoethanol (ß-ME) or dithiothreitol (DTT). To promote reduction of disulfide bonds, add 10 µl ß-ME per 1 ml of buffer RL. Upon addition of ß-ME, RL buffer remains stable for 1 month. A less toxic but more expensive alternative to ß-ME is DTT, to add 10 µl of [1 M] DTT per 1 ml buffer RL before use. DTT is not stable in RL buffer, thus DTT-supplemented RL buffer aliquots must not be stored. Working aliquots of [1 M] DTT stock solution in RNase free water must be stored at -20°C for maintaining stability. To set up a [1 M] DTT stock solution (MW = 154.25 g mol^{-1}), dissolve 1.54 g DTT per 10 ml RNase free water and store in aliquots for one-time usage.

Equipment and reagents to be supplied by the user

- For all protocols:

  Magnetic stand E0361 for 16 tubes, E0362 for 24 tubes, E0363 for 96-well plate. To be purchased separately.

  ethanol 96–100%, β-mercaptoethanol (14.3 M, β-ME) or [1 M] Dithiothreitol (DTT) in RNase-free water

  Disposable gloves, pipets, sterile pipet tips, sterile RNase-free 1.5-2 ml tubes or 96-well plates (well volume at least 800 µl), vortex and laboratory rack for the tubes.

- For bacteria protocol – lysozyme, TE buffer (10 mM Tris pH 7.5, 1 mM EDTA).
- For tissue protocol – equipment for sample disruption and homogenization, depending on the method chosen: mortar and pestle and liquid nitrogen or handheld rotor-stator homogenizer.
- For yeast protocol – BeadTubeDry Cat. no E0358, PBS buffer (Cat. no E0281) or 0.9 % NaCl. Optional buffer YL: 1 M sorbitol, 0.1 M EDTA, lyticase/zymolase.
- For blood protocol - Lyse RBC buffer (Cat. no. E0326) for erythrocytes lysis. When the blood volume exceeds 400 µl – appropriate size plastic tubes for erythrocytes lysis and centrifugation after lysis.

Protocol

Part I Before starting

1. Dissolve **DNase I** by adding **DNase I buffer** to obtain a solution of 5U/µl.

   - *96 preps kit contains 4 tubes with freeze-dried DNase I (275 U each). Add 55 µl of DNase I buffer to each DNase I tube and incubate for 1 min in room temperature. Gently swirl the tube to completely dissolve the DNase I.*
   - *Be careful not to mix DNase I virgously as this enzyme is sensitive to mechanical agitation.*
   - *Store dissolved DNase I in -20°C.*

Part II Samples preparation

Tissues

NOTE 1 • This protocol is designed for isolation of total RNA from animal tissues.

NOTE 2 • If using mortar and pestle for homogenization sample, do not use more than 30 mg tissues. If using rotor-stator homogenizer use up to 10 times less tissues.

NOTE 3 • Frozen animal tissue should not be allowed to thaw during handling.

NOTE 4 • Add 10 µl β-mercaptoethanol (β-ME) or 10 µl DTT [1 M] per 1 ml buffer RL before use. Example: For two RNA preparations, mix 800 µl (2 x 400 µl) buffer RL with 8 µl β-ME. Buffer RL is stable for 1 month after addition of β-ME. DTT is not stable within buffer RL (see page 3, NOTE 6).

1. Choose homogenization method:

   a) Grind animal tissue under liquid nitrogen to a fine powder using previously cooled mortar and pestle. Place sample material in liquid nitrogen-cooled 2 ml Eppendorf tube.

      - *To obtain high yield of RNA a tissue fragment should be thoroughly grinded to a fine powder.*
      - *Frozen tissue should not be allowed to thaw during handling.*
      - *Do not use more than 30 mg tissues.*

   b) Place the weighed tissue (fresh or frozen) in a suitably sized vessel for homogenizer. Add 400 µl buffer **RL**. Homogenize using conventional rotor-stator homogenizer until the sample is homogeneous. Continue the protocol with step 3.

      - *If using mortar and pestle, do not use more than 30 mg tissues. If using rotor-stator homogenizer use up to 10 times less tissues.*
      - *We recommend homogenization with rotor-stator homogenizer for maximum total RNA yields.*

2. Add 400 µl buffer **RL** to a tissue powder. Mix thoroughly by vigorous vortexing.
3. Centrifuge sample for 3 min at maximum speed.
4. Carefully transfer the supernatant to the new 1.5 ml tube or well (for further processing on 96-well plate) and add 0.7 volumes of ethanol (96-100%) and mix thoroughly by pipetting.
   - *For example, if 400 µl supernatant was recovered add 280 µl ethanol.*
5. Continue the protocol with step 1 Part III Isolation and purification of RNA.

Bacteria

NOTE 1 · This protocol is designed for isolation of total RNA from any Gram+ and Gram- bacteria.

NOTE 2 · Prepare TE buffer with 500 µg/ml lysozyme for Gram- bacteria or 5 mg/ml lysozyme for Gram + bacteria.

NOTE 3 · Add 10 µl β-mercaptoethanol (β-ME) or 10 µl DTT [1 M] per 1 ml buffer RL before use. Example: For two RNA preparations, mix 700 µl (2 x 350 µl) buffer RL with 7 µl β-ME. Buffer RL is stable for 1 month after addition of β-ME. DTT is not stable within buffer RL (see page 3, NOTE 6).

NOTE 4 · The bacterial culture should be harvested at 4°C. All subsequent steps of the protocol should be performed at room temperature.

1. Pellet bacteria from overnight culture by centrifugation (for 5 min at 4°C) and discard the supernatant, ensuring that all liquid is completely removed.
   - *Do not use more than $1x10^9$ bacteria.*
   - *The highest quality RNA is obtained from bacterial culture, which are either in log phase or early stationary phase.*
2. Resuspend the bacterial pellet in 100 µl lysozyme-containing TE buffer (see note 2). Mix by vortexing.
3. Incubate the sample at room temperature for:
   a) 5–10 min gram-negative bacteria
   b) 15–20 min gram-positive bacteria
4. Add 350 µl buffer **RL** to the sample. Mix thoroughly by vigorous vortexing.
5. Centrifuge sample for 3 min at maximum speed.
6. Carefully transfer the supernatant to the new 1.5 ml tube or well (for further processing on 96-well plate) and add 0.7 volumes of ethanol (96-100%) and mix thoroughly by pipetting.
   - *For example, if 400 µl supernatant was recovered add 280 µl ethanol.*
7. Continue the protocol with step 1 Part III Isolation and purification of RNA.

Yeast

NOTE 1 • This protocol is designed for isolation of total RNA from yeast.

NOTE 2 • Efficient homogenization of yeast cells is the most crucial step for the high yield and good quality RNA purification. Yeast cell wall can be easily lysed by grinding by glass beads BeadTubeDry Cat. no E0358 in a process of shaking/vortexing. The procedure allows for high yield, enzyme-free RNA purification from yeast.

NOTE 3 • Add 10 µl β-mercaptoethanol (β-ME) or 10 µl DTT [1 M] per 1 ml buffer RL before use. Example: For two RNA preparations, mix 700 µl (2 x 350 µl) buffer RL with 7 µl β-ME. Buffer RL is stable for 1 month after addition of β-ME. DTT is not stable within buffer RL (see page 3, NOTE 6).

NOTE 4 • GeneMAGNET RNA Purification Kit Cat. no E3401 does not include BeadTubeDry Cat. no E0358. BeadTubeDry can be purchased separately.

NOTE 5 • The yeast culture should be harvested at 4°C. All subsequent steps of the protocol should be performed at room temperature.

NOTE 6 • Prepare PBS or 0.9 % NaCl solution. PBS buffer can be purchased separately Cat. no E0281.

NOTE 7 • If enzymatic cell wall lysis is required use alternative RNA purification protocol: Yeast - enzymatic lysis (page 9).

1. Harvest yeast cells by centrifugation at 5 000 x g for 5 min at 2-8°C and discard the supernatant, ensuring that all liquid is completely removed.

   - *Do not use more than 5 x10^7 yeast cells.*

2. Resuspend cells in 100 µl PBS of 0,9 % NaCl and transfer to the BeadTubeDry.

   - *Use only freshly harvested cells.*

3. Secure BeadTubeDry horizontally to a vortex by using a vortex adapter or a tube holder. Vortex at maximum speed for 5 min. Do not allow for heating the tubes. After vortexing place the tubes on ice.

   - *Cell disruptor (FastPrep, Precellys, Disruptor Genie, etc.) shall preferably be used. Maximum DNA yields are achieved by using a cell disruptor rather than by vortexing. But, for preventing heating the cells, it is required to optimize the shaking time (generally, a time reduction, as compared to the time specified above for vortexing, depending on the specific type of cell disruptor in use).*

4. Add 350 µl **RL** into BeadTubeDry and mix by vortexing.

5. Centrifuge sample for 1 min at maximum speed.

6. Carefully transfer the supernatant to the new 1.5 ml tube or well (for further processing on 96-well plate) and add 0.7 volumes of ethanol (96-100%) and mix thoroughly by pipetting.
   - *For example, if 400 µl supernatant was recovered add 280 µl ethanol.*
7. Continue the protocol with step 1 Part III Isolation and purification of RNA.

Yeast - enzymatic lysis

NOTE 1 • This alternative protocol is designed for isolation of total RNA from yeast.

NOTE 2 • Prepare buffer YL 1 M sorbitol, 0.1 M EDTA. Just before use, add: 50 U lyticase/zymolase per 1 x 10^7 cells and either 0.1% β-mercaptoethanol or 0.1% DTT.

NOTE 3 • Add 10 µl β-mercaptoethanol (β-ME) or 10 µl DTT [1 M] per 1 ml buffer RL before use. Example: For two RNA preparations, mix 700 µl (2 x 350 µl) buffer RL with 7 µl β-ME or DTT. Buffer RL is stable for 1 month after addition of β-ME. DTT is not stable within buffer RL (see page 3, NOTE 6).

NOTE 4 • The yeast should be harvested at 2–8°C. After harvesting the cells, all centrifugation steps should be performed at room temperature. Use only freshly harvested cells.

1. Harvest yeast cells by centrifugation at 5 000 x g for 5 min at 2–8°C and discard the supernatant, ensuring that all liquid is completely removed.
   - *Do not use more than 5 x10^7 yeast cells.*
2. Resuspend cells in 1.5 ml lyticase/zymolase-containing buffer YL (see NOTE 2). Incubate for 30 min at 30°C to generate spheroplasts.
   - *Use only freshly harvested cells.*
3. Pellet the resulting spheroplasts by centrifugation for 5 min at 1 000 x g. Carefully remove the supernatant.
4. Add 350 µl buffer **RL** to lyse pelleted spheroplasts. Mix thoroughly by vigorous vortexing.
   - *Optional: transfer the sample to the 96 well plate for further processing.*
5. Add 0.7 volumes of ethanol (96-100%) and mix thoroughly by pipetting.
   - *For example, if 400 µl supernatant was recovered add 280 µl ethanol.*
6. Continue the protocol with step 1 Part III Isolation and purification of RNA.

Cell Culture

NOTE 1 • This protocol is designed for isolation of total RNA from cell culture.

NOTE 2 • Do not use more than $5x10^6$ cells.

NOTE 3 • Add 10 µl β-mercaptoethanol (β-ME) or 10 µl DTT [1 M] per 1 ml buffer RL before use. Example: For two RNA preparations, mix 800 µl (2 x 400 µl) buffer RL with 8 µl β-ME. Buffer RL is stable for 1 month after addition of β-ME. DTT is not stable within buffer RL (see page 3, NOTE 6).

1. Pellet cells by centrifugation in the 2 ml Eppendorf tube for 5 min at 1 000 x g and remove media.
2. Add 400 µl buffer **RL** to the cell pellet. Pipette the cell lysate several times to ensure sufficient cell disruption.
   - *Optional: transfer the sample to the 96 well plate for further processing.*
3. Add 0.7 volumes of ethanol (96-100%) and and mix thoroughly by pipetting.
4. Continue the protocol with step 1 Part III Isolation and purification of RNA.

Blood (leukocytes)

NOTE 1 • This protocol is designed for isolation of total RNA from fresh human blood. The kit is not suitable for isolation of RNA from frozen blood. For RNA isolation from stored or frozen blood samples, use the spin-column GeneMATRIX Universal Blood RNA Purification Kit (Cat. no. E3594). In this case the blood must be stabilized with additional buffer included in the spin-column kit. After freezing, the blood is not suitable for isolation of RNA using this protocol.

NOTE 2 • After erythrocytes lysis (step 5), leukocytes can be stored in Fix RNA solution (Cat. no E0280).

NOTE 3 • Add 10 µl β-mercaptoethanol (β-ME) or 10 µl DTT [1 M] per 1 ml buffer RL before use. Example: For two RNA preparations, mix 700 µl (2 x 350 µl) buffer RL with 7 µl β-ME or DTT. Buffer RL is stable for 1 month after addition of β-ME. DTT is not stable within buffer RL (see page 3, NOTE 6).

NOTE 4 • GeneMAGNET RNA Purification Kit does not contain Lyse RBC buffer. Lyse RBC can be purchased separately (Cat. no E0326).

1. Add 4 volumes of buffer Lyse RBC to a fresh blood. Mix by inverting the tube.
   - *For example, if the starting blood volume is 300 µl, add 1200 µl of Lyse RBC buffer.*
2. Keep at 4°C for 10 min to lyse erythrocytes. Mix twice by inverting the tube.
3. Centrifuge at 400 x g for 10 min at 4°C, and carefully decant the supernatant.
   - *Carefully pipette to collect the rest of the supernatant.*
4. Add two volumes of Lyse RBC to the leukocytes pellet. Mix thoroughly by vigorous vortexing.
   - *For example, if the starting blood volume is 300 µl, add 600 µl of Lyse RBC buffer.*
5. Centrifuge at 400 x g for 10 min at 4°C, and carefully decant the supernatant.
   - *Carefully pipette to collect the rest of the supernatant.*
6. Add 400 µl buffer **RL** to the leukocytes pellet. Mix thoroughly by pipetting for homogenization.
   - *Optional: transfer the sample to the 96 well plate for further processing.*
7. Add 0.7 volumes of ethanol (96-100%) and and mix thoroughly by pipetting.
8. Continue the protocol with step 1 Part III Isolation and purification of RNA.

Part III Isolation and purification of RNA

Nucleic acids isolation can be performed in 1.5-2 ml Eppendorf tubes or on 96-well plates with working volume 800 µl.

1. Resuspend **Magnetic Beads** before removing them from the storage tube by vortexing. Add 20 µl of resuspended **Magnetic Beads** to the sample and mix by vortexing or pipeting for 1 min.
2. Separate the magnetic beads against the side of the tubes/wells. Wait until all the beads have been attached to the magnets.
3. Remove and discard the supernatant by pipeting. Remove the magnetic stand/transfer tubes to the laboratory rack, add 300 µl of **Wash RBW** and mix by pipeting or vortexing for 10 s.
4. Separate the magnetic beads against the side of the tubes/wells. Wait until all the beads have been attached to the magnets.
5. Remove and discard the supernatant by pipeting. Remove the magnetic stand/transfer tubes to the laboratory rack, add 98 µl **DNR** and 2 µl **DNase I** (5 U/µl), resuspend **Magnetic Beads** in the solution by gentle pipeting and incubate 15 min in the room temperature. Add 500 µl ethyl alcohol 96-100 % into the sample and mix thoroughly for 1 min.

   - *Be sure to resuspend Magnetic Beads in DNase I solution to enable efficient DNA digestion.*
   - *The addition of ethyl alcohol into the sample after DNA digestion is crutial for rebinding of RNA to Magnetic Beads.*

6. Separate the magnetic beads against the side of the tubes/wells. Wait until all the beads have been attached to the magnets.
7. Remove and discard the supernatant by pipeting. Remove the magnetic stand/transfer tubes to the laboratory rack, add 300 µl of **Wash RBW** and mix by pipeting or vortexing for 10 s.
8. Separate the magnetic beads against the side of the tubes/wells. Wait until all the beads have been attached to the magnets.
9. Remove and discard the supernatant by pipeting. Remove the magnetic stand/transfer tubes to the laboratory rack, add 300 µl of **Wash RBW2** and mix by pipeting or vortexing for 10 s.
10. Separate the magnetic beads against the side of the tubes/wells. Wait until all the beads have been attached to the magnets.

11. Remove and discard the supernatant by pipeting. Leave the open tubes/plate in magnetic stand and air dry the beads for 15-20 min.

    - *Wash RBW2 contains alcohol, make sure all the solution evaporates before proceding to step 12.*

12. Add 50-100 µl **RNase-free water** to the tube/well and mix by pipeting or vortexing for 2-5 min.

    - *Be sure to resuspend Magnetic Beads thoroughly.*

13. Separate the magnetic beads against the side of the wells. After all the beads have been attached to the magnets transfer the supernatant containing the purified RNA to a suitable tube/plate. RNA is ready for analysis/manipulations. Isolated RNA can be stored either at 2-8°C (preferred) or at -20°C. Avoid multiple freezing and thawing.

Safety Information

RL

Warning

H302+**H332** Harmful if swallowed or if inhaled.
H412 Harmful to aquatic life with long lasting effects.
P273 Avoid release to the environment.
P301+**P312** If swallowed: call a poison center/ doctor/... if you feel unwell.
P304+**P340** If inhaled: remove person to fresh air and keep comfortable for breathing.
EU**H032** Contact with acids liberates very toxic gas.

Wash RBW

Danger

H225 Highly flammable liquid and vapour.
H319 Causes serious eye irritation.
P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P305+**P351**+**P338** If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P403+**P235** Store in a well-ventilated place. Keep cool.
P337+**P313** If eye irritation persists: Get medical advice/ attention.

Wash RBW2

Danger

H225 Highly flammable liquid and vapour.
H319 Causes serious eye irritation.
P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
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P403+**P235** Store in a well-ventilated place. Keep cool.
P337+**P313** If eye irritation persists: Get medical advice/ attention.

- ***GeneMAGNET RNA Purifictation Kit is designed for rapid, thorough isolation and purification of total RNA from a broad variety of sources: animal and human tissues, fungi, cell cultures, bacteria and yeast cells or blood (leukocytes), among others.***

  Purified RNA is free of contaminants like proteins, nucleases, and other impurities. Samples are first lysed in the presence of a denaturing buffer. Upon lysis buffer addition, cellular RNases are immediately inactivated. The procedure includes enzymatic DNA digestion, the addition of etanol into the lysate enables selective RNA binding onto Magnetic Beads. Prior to elution by RNase-free water, RNA is washed several times. The obtained RNA isolate is ready for downstream application like reverse transcription, RT-qPCR or PCR.

- **GeneMAGNET line is based on the use of silica paramagnetic beads (Magnetic Beads) for selective binding of RNA and DNA. The use of specially designed binding and washing buffers enables the efficient purification of highly pure nucleic acids.**

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