EURx® MOLECULAR BIOLOGY PRODUCTS

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GeneMATRIX Universal DNA/RNA/Protein Purification Kit

Universal kit for isolation of genomic DNA, total RNA and total protein from the same biological sample from bacteria, tissue, plant, yeast and cell culture.

Cat. no. E3597

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Introductory Notes

NOTE 1 · This kit is designed for isolation of genomic DNA, total RNA, and total protein simultaneously from a single biological sample.

NOTE 2 · The kit is designed to purify DNA/RNA/Protein from a bacteria, tissue, plant, yeast or cell culture.

NOTE 3 · DNA binding capacity is 30 µg per spin-column. Loading more than 30 µg DNA may lead to DNA contamination of the RNA eluate. The maximum volume of the column reservoir is 650 µl.

NOTE 4 · The RNA binding capacity is 100 µg per spin-column. The maximum volume of the column reservoir is 650 µl.

NOTE 5 · Avoid overloading the mini columns. Overloading will significantly reduce yield and purity and may block the mini columns.

NOTE 6 · 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 DRP before use. Upon addition of ß-ME, DRP buffer remains stable for 1 month. A less toxic but more expensive alternative to ß-ME is, to add 10 µl of [1 M] DTT in RNase free water per 1 ml buffer DRP before use. DTT is not stable in buffer DRP, thus DTT-supplemented DRP 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.

NOTE 7 · The procedure employs our DNA-binding spin-columns, which removes DNA by binding to the resin. In addition, washing steps with specifically optimized buffer Wash DN1 effectively eliminates any remaining DNA. However, for RNA applications that are very sensitive to traces of DNA, use Appendix 1 (page 11) with optional on-column DNase digestion. In this case omit the use of buffer Wash RB1 during the DNA purification procedure (part IV of the Protocol point 1) and use this solution during the purification of RNA with on-column DNase digestion (Appendix 1, point 3).

NOTE 8 · Per 1 ml buffer Lyse ALL, add 10 µl β-mercaptoethanol (β-ME) or [1 M] Dithiothreitol (DTT) in RNase free water before use. Lyse ALL is stable for 1 month after addition of β-ME. DTT is not stable in buffer Lyse ALL.

NOTE 9 · Add 10 µl **Bromophenol Blue** and either 25 µl β-mercaptoethanol (β-ME) or [1 M] Dithiothreitol (DTT) in RNase free water and per 1 ml buffer PLB before use. After addition of β-ME store buffer PLB at 2–8°C.

NOTE 10 · Certain bacterial species are resistant to lysis, thus supplementary enzymes other than lysozyme may be necessary. For example, lysis of Staphylococcus is much more efficient with lysostaphin.

NOTE 11 · For efficient lysis of yeast species zymolase or lyticase is necessary. Alternatively, mechanical lysis with the use of glass beads (BeadTubeDry E0358) and bead beater device can be employed to break yeast cell walls.

NOTE 12 · All solutions should be kept tightly closed to avoid evaporation and resulting components concentration changes. Store all components of the kit at 15–25°C with the exception of DNA Binding Columns and RNA Binding Columns, which should be kept at 2–8°C.

NOTE 13 · Elution buffer is a low salt solution, that contains no metal ion chealators (e.g. EDTA) that can inhibit subsequent enzymatic reactions. Elution buffer composition is suitable for downstream applications such as digestion with restriction enzymes, phosphorylation, ligation, Sanger sequencing, NGS etc. It is also possible to elute the DNA with Tris-HCl, water or TE.

Content	25 preps E3597-01	100 preps E3597-02	Storage/Stability
Lyse ALL	6 ml	24 ml	15-25°C
DRP	15 ml	60 ml	15-25°C
Wash DN1	12 ml	48 ml	15-25°C
Wash RB1	12 ml	48 ml	15-25°C
Wash RBW	54 ml	216 ml	15-25°C
DNR	1.5 ml	6 ml	15-25°C
PLB	4.5 ml	18 ml	15-25°C
Bromophenol Blue 0.5%	0.1 ml	0.4 ml	15-25°C
RNase-free water	3 ml	12 ml	15-25°C
Elution	3 ml	12 ml	15-25°C
DNA Binding Columns	25	2 x 50	2-8°C
RNA Binding Columns	25	2 x 50	2-8°C
Protocol	1	1	

Equipment and reagents to be supplied by user:

1. For all protocols: Either β-mercaptoethanol (14.3 M, β-ME) or [1 M] Dithiothreitol (DTT) in RNase free water, ethanol 96–100%, microcentrifuge, disposable gloves, sterile RNase-free pipet tips, sterile RNase-free 1.5–2 ml tubes.
2. Optional **DNase I** for on-column digestion (Cat. no. E1345; see Appendix 1, page 11).
3. For bacteria protocol – lysozyme.
4. For tissue and plant protocol – equipment for sample disruption and homogenization, depending on the method chosen: mortar and pestle and liquid nitrogen or handheld rotor-stator homogenizer.

5. Optional for tissue and plant protocol: antifoaming reagent for EURx lysis buffers **AFR01** (Cat. no. E0328). While using rich in detergents solutions (**Lyse ALL**) excessive foaming may occur. This is particularly visible when using mechanical homogenizers or when samples are shaken with different types of beads. This foaming is substantially reduced by adding **AFR01** reagent to lysis buffers at a final concentration of 0.5% (v/v) before starting disruption and homogenization (add 5 µl to 1 ml lysis buffer).
6. For yeast protocol – buffer **SE**: 1 M sorbitol, 0.1 M EDTA, and lyticase (E0329) or BeadTubeDry (E0358).
7. For Appendix 2 (page 12): RNA Purification of plant tissues rich in phenolic compounds and lignins - Lyse Buffer PVP (E0291-01).

Protocol

Part I Disruption, lysis, homogenization and DNA binding

Animal tissue

1. **a)** Grind animal tissue under liquid nitrogen to a fine powder using previously cooled mortar and pestle. Place sample material in RNase-free, cooled 2 ml Eppendorf tube. Add 200 µl **Lyse ALL** and 300 µl **DRP** buffer to a tissue powder. Mix thoroughly by vortexing vigorously.

   b) Place the weighed tissue (fresh or frozen) in a suitably sized vessel for homogenizer. Add 300 µl **DRP** buffer and homogenize using conventional rotor-stator homogenizer until the sample is homogeneous. Add 200 µl **Lyse ALL** to the homogenized sample. Mix thoroughly.

   - *If using mortar and pestle, do not use more than 20 mg tissues. If using rotor-stator homogenizer use up to 10 times less tissues. We recommend homogenization with rotor-stator homogenizer for maximum total DNA/RNA yields.*
   - *To obtain high yield of DNA/RNA a tissue fragment should be thoroughly grinded to a fine powder.*
   - *Frozen tissue should not be allowed to thaw during handling.*
   - *Ensure that either β-ME or DTT is added to buffers DRP and Lyse ALL (see page 3, notes 6 and 8).*
2. Centrifuge sample for 3 min at maximum speed.
3. Carefully transfer the supernatant to the **DNA binding spin-column** placed in a 2 ml collection tube. Centrifuge at 12 000 x g for 1 min.
4. Store the **DNA binding spin-column** at room temperature 15–25°C or at 2–8°C for later DNA purification (part IV of the protocol, page 10). Use the flow-through for RNA purification. Continue immediately with part II, point 1 of the protocol (RNA isolation, page 8).

Cell culture

1. Centrifuge the cell culture in the 2 ml Eppendorf tube for 5 min at 1000 x g .
   - *o not use more than 5x10^6 cells.*
2. Add 400 µl buffer **DRP** to the cell pellet. Mix thoroughly by vigorous vortexing and pipetting for homogenization.
   - *It is possible to use rotor-stator for homogenization cells at this step. Homogenization with rotor-stator generally results in higher genomic DNA/RNA yields. Homogenize cells for 30 s in 400 µl DRP buffer per prep using a rotor-stator homogenizer.*
   - *Ensure that either β-ME or DTT is added to buffer DRP (see page 3, note 6).*
3. Add 100 µl **Lyse ALL** to the homogenized sample. Mix thoroughly.
   - *Ensure that either β-ME or DTT is added to buffer Lyse ALL (see page 3, note 7).*
4. Centrifuge sample for 2 min at maximum speed.
5. Carefully transfer the supernatant to the **DNA binding spin-column** placed in a 2 ml collection tube. Centrifuge at 12 000 x g for 1 min.
6. Store the DNA binding spin column at room temperature 15–25°C or at 2–8°C for later DNA purification (part IV of the protocol, page 10). **Use the flow-through for RNA purification**. Continue immediately with part II, point 1 of the protocol (RNA isolation, page 8).

Plant

1. **a)** Grind plant tissue under liquid nitrogen to a fine powder using previously cooled mortar and pestle. Place sample material (max. 100 mg) in RNase-free, cooled 2 ml Eppendorf tube. Add 200 µl **Lyse ALL** and 100 µl **DRP** buffer to a plant tissue powder. Mix thoroughly by vortexing vigorously.
   - *In the case of plants with high phenolic compounds level, lignins accumulation or problems with downstream reactions (PCR/ RT-qPCR), it is recommended to exchange Lyse ALL buffer with Lyse Buffer PVP cat. no E0291-01 and proceed with Appendix 2 (page 12).*

   b) Place the weighed plant tissue (fresh or frozen) in a suitably sized vessel for homogenizer. Add 200 µl **Lyse ALL** and 100 µl **DRP** buffer and homogenize using conventional rotor-stator homogenizer until the sample is homogeneous.
   - *If using mortar and pestle, do not use more than 100 mg plant tissues. If using rotor-stator homogenizer use up to 10 times less plant tissues. We recommend homogenization with rotor-stator homogenizer for maximum total RNA yields.*
   - *To obtain high yield of DNA/RNA a tissue fragment should be thoroughly grinded to a fine powder.*
   - *Frozen plant tissue should not be allowed to thaw during handling.*
   - *Ensure that either β-ME or DTT is added to buffers DRP and Lyse ALL (see page 3 notes 6 and 8).*
2. Centrifuge sample for 4 min at maximum speed.
3. Carefully transfer the supernatant to the new, RNase-free, Eppendorf tube and incubate on ice for 10 min.

4. Centrifuge sample for 4 min at maximum speed.
5. Carefully transfer the supernatant to the new, RNase-free, Eppendorf tube and add 200 µl **DRP** buffer.
6. Carefully transfer the supernatant to the **DNA binding spin-column** placed in a 2 ml collection tube. Centrifuge at 12 000 x g for 1 min.
7. Store the DNA binding spin column at room temperature 15–25°C or at 2–8°C for later DNA purification (part IV of the protocol, page 10). Use the flow-through for RNA purification. Continue immediately with part II, point 1 of the protocol (RNA isolation, page 8).

Yeast

1. Harvest yeast cells by centrifugation at 5000 x g for 5 min at 4°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 500 µl lyticase-containing buffer **SE** (see note 3 below). Incubate for 30 min at 30°C.
   - *For high yield isolation it is critical to completely resuspend yeast cells.*
   - *Due to the different growth characteristics of yeast species, performing a preliminary experiment to determine the optimal starting volume is recommended. Weight of pellet should not exceed 100 mg per one minicolumn.*
   - *Prepare buffer SE: 1 M sorbitol, 0.1 M EDTA. Just before use, add 10 µl (50 U) lyticase E0329 per 1 x 10^7 cells and either 0.1% β-mercaptoethanol or 0.1% DTT.*
   - *Alternatively, mechanical lysis with the use of glass beads (BeadTubeDry E0358) and bead beater device can be employed to break yeast cell walls. The force of mechanical lysis should be adjusted to different yeast species.*
3. Pellet the spheroplasts at 300 x g (app. 3000 rpm) for 3 min. Discard the supernatant.
4. Add 200 µl **Lyse ALL** and 350 µl **DRP** buffer to the sample and mix thoroughly by pipetting and vortexing vigorously.
   - *Ensure that either β-ME or DTT is added to buffers DRP and Lyse ALL (see page 3, notes 6 and 8).*
5. Centrifuge sample for 2 min at maximum speed.
6. Carefully transfer the supernatant to the **DNA binding spin-column** placed in a 2 ml collection tube. Centrifuge at 12 000 x g for 1 min.
7. Store the DNA binding spin column at room temperature 15–25°C or at 2–8°C for later DNA purification (part IV of the protocol, page 10). Use the flow-through for RNA purification. Continue immediately with part II, point 1 of the protocol (RNA isolation, page 8).

Bacteria

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 DNA is obtained from bacterial culture, which are either in log phase or early stationery phase.*

2. Resuspend the bacterial pellet in 200 µl lysozyme-containing **Lyse ALL** buffer. Mix by vortexing.
   - *Add lysozyme to the Lyse ALL buffer: 500 µg/ml lysozyme for Gram- bacteria or 5 mg/ml lysozyme for Gram+ bacteria.*
   - *Ensure that either β-ME or DTT is added to buffer Lyse ALL (see page 3, note 8).*
3. Incubate the sample at room temperature for:
   a) 5–10 min gram-negative bacteria
   b) 15–20 min gram-positive bacteria
4. Add 300 µl buffer **DRP** to the sample. Mix thoroughly by vortexing vigorously.
   - *Ensure that either β-ME or DTT is added to buffer DRP (see page 3, note 6).*
5. Centrifuge sample for 2 min at maximum speed.
6. Carefully transfer the supernatant to the **DNA binding spin-column** placed in a 2 ml collection tube. Centrifuge at 11 000 x g for 1 min.
7. Store the DNA binding spin column at room temperature 15–25°C or at 2–8°C for later DNA purification (part IV of the protocol, page 10). **Use the flow-through for RNA purification**. Continue immediately with part II, point 1 of the protocol (RNA isolation, page 8).

Part II RNA isolation

1. To the flow-through from DNA binding step (last step in part I of the protocol), add 0.7 volumes of ethanol (96–100% [v/v]). Mix thoroughly by pipetting. Do not centrifuge.
   - *For example, if 400 µl supernatant was recovered in last step in Part I of the protocol, add 280 µl ethanol.*
   - *A precipitate may form after addition of ethanol.*
2. Apply up to 600 µl of a mixture, including any precipitate, to the **RNA binding spin-column** placed in a 2 ml collection tube. Centrifuge for 1 min at 11 000 x g. Transfer the flow-through to a 2 ml tube for protein purification (part III of the protocol, page 9).
3. Transfer the remaining mixture to the same **RNA binding spin-column** and centrifuge at 11 000 x g for 1 min. Remove the spin-column, pour off supernatant and place back into the receiver tube.
4. Add 400 µl of **Wash DN1** buffer to the **RNA binding spin-column** and centrifuge at 11 000 x g for 1 min. Remove the spin-column, pour off supernatant and place back into the receiver tube.
   - *This step effectively eliminates remaining DNA. However, for RNA applications that are very sensitive to traces amounts of DNA, in the next step use Appendix 1 (page 11) with optional on-column DNase digestion.*

5. Add 600 µl of **Wash RBW** buffer and spin down at 11 000 x g for 1 min.
6. Remove the spin-column, pour off supernatant and place back into the receiver tube.
7. Add 300 µl **Wash RBW** buffer and spin down at 11 000 x g for 2 min.

   - *Be careful not to contaminate the sample while removing spin-column from receiver tube. Check whether membrane is completely dry. If not, pour off supernatant and place back spin-column into the receiver tube and spin down for additional 1 min.*

8. Place spin-column into new receiver tube (1.5–2 ml) and add 50–100 µl **RNase-free water** directly onto the membrane.
9. Centrifuge for 1 min at 11 000 x g.
10. Remove spin-column, cap the receiver tube. RNA is ready for analysis/manipulations. Store the samples at -20°C or below.

Part III Protein precipitation

1. To the flow-through from RNA binding step (step 2 in part II of the protocol), add 2 volumes of ethanol (96–100%). Mix thoroughly. Incubate at 2–8°C for 30 min.
2. Centrifuge at maximum speed for 20 min at 4°C, and carefully decant the supernatant.
3. Add 300 µl of 70% ethanol to the protein pellet. Vortex well and centrifuge at maximum speed for 10 min at 4°C, remove the supernatant.
4. Dry the protein pellet for 5–15 min at room temperature.
5. Dissolve the protein pellet in 80–150 µl protein loading buffer **PLB** (Note 9 page 3).

   - *Buffer PLB is a sample buffer for use in SDS-PAGE analysis. If the proteins will not be analyzed by SDS-PAGE, use a buffer compatible with the intended application. As a result of the method of isolation the precipitated protein is highly denatured and shows reduced solubility in water. Dissolution the precipitate is possible in PLB buffer or other solution containing a high concentration of detergent (eg 1.5–5% SDS). Therefor, Bradford and Lowry assays are not applicable for quantifying protein yield. For protein quantitation, use the Bicinchoninic Acid Assay (BCA).*

   - *For SDS-PAGE analysis, add 10 µl Bromophenol Blue and either 25 µl β-mercaptoethanol (β-ME) or 25 µl [1 M] dithiothreitol per 1 ml buffer PLB before use. After addition of β-ME store buffer PLB at 2–8°C. DTT-supplemented buffer PLB must not be stored, always prepare fresh aliquots.*

   - *In case of PLB buffer ingredients precipitation warm up until clarified.*

6. Incubate for 5 min at 95°C to dissolve and denature sample.
7. If some insoluble material is still visible, centrifuge at maximum speed for 1 min. The supernatant is ready to use in downstream applications such us SDS-PAGE and others.

   - *Sample can be stored at 2–8°C for short period or at -20°C for several months.*

Part IV Genomic DNA purification

1. Add 400 µl of **Wash RB1** buffer to the DNA binding spin column (from the last step of part I of the protocol) and centrifuge at 11 000 x g for 1 min.

   In the case of purification of RNA with on-column DNase digestion skip this step and proceed to wash using the Wash RBW.

2. Remove the spin-column, pour off supernatant and place back into the receiver tube.
3. Add 500 µl of **Wash RBW** buffer and spin down at 11 000 x g for 1 min.
4. Remove the spin-column, pour off supernatant and place back into the receiver tube.
5. Add 300 µl of **Wash RBW** buffer and spin down at 11 000 x g for 1 min.

   Be careful not to contaminate the sample while removing spin-column from receiver tube. Check whether membrane is completely dry. If not, pour off supernatant and place back spin-column into the receiver tube and spin down for additional 1 min.

6. Place spin-column into new receiver tube (1.5–2 ml) and add 50–100 µl of **Elution** buffer directly onto the membrane to elute bound DNA.

   Addition of the elution buffer directly onto the center of the resin improves DNA yield. To avoid transferring traces of DNA between the spin-columns do not touch the spin-column walls with the micro-pipette.

   In order to improve the efficiency of the elution genomic DNA from membrane, Elution buffer can be heated to a temperature of 80°C.

7. Incubate spin-column/receiver tube assembly for 2 min at room temperature.
8. Centrifuge for 1 min at 11 000 x g.
9. Remove spin-column, cap the receiver tube. Genomic DNA is ready for analysis/ manipulations. It can be stored either at 2–8°C (preferred) or at -20°C (avoid multiple freezing and defrosting of DNA).

Appendix 1

RNA Purification with On-Column DNase digestion

NOTE 1 · Use this protocol after step with Wash DN1 in standard procedure in Part II RNA isolation.

NOTE 2 · Perform on-column DNase digestion only by using buffer DNR, which ships with the kit. Other DNase buffers are not compatible with on-column DNase digestion.

NOTE 3 · DNase I is not supplied with this kit, but is available as a separate product (Cat. no. E1345).

NOTE 4 · Prepare DNase I solution before starting this procedure. Add 1–2 U (Kunitz) of DNase I per 50 µl DNR buffer. Do not add more than 2 µl DNase I solution per 50 µl DNR buffer. Dissolve solid DNase I in the storage buffer (50 mM Tris-acetate pH 7.5, 10 mM $CaCl_2$ and 50% v/v glycerol) in a concentration of 1–2 U/µl (Kunitz) and then add 1 µl DNase I per 50 µl DNR buffer.

NOTE 5 · DNase I is sensitive to physical denaturation. Be careful not to mix DNase vigorously.

NOTE 6 · Use only RNase-free DNase I preparations of high quality.

NOTE 7 · Commercially available RNase-free DNase I preparations vary strongly with respect to their behaviour towards RNA. During routine quality controls, we noted that many commercially available RNase-free DNase I preparations still exhibit residual detectable RNase activity, as visually detectable by band retardations in PAGE gel electrophoresis. Therefore we strongly recommend using EURx RNase-free DNase I (Cat. no. E1345), which does not exhibit residual RNase activity.

1. After the step with **Wash DN1** and centrifugation remove the spin-column, pour off supernatant and place back into the receiver tube.
2. Add 50 µl **DNR** buffer, with **DNase I** added, directly onto the membrane and place on the benchtop at room temperature for 10-20 min. Do not centrifuge.
   - *Ensure that DNase I is added to buffer DNR. See note 4 above.*
3. Add 400 µl **Wash RB1** buffer and spin down at 11 000 x g for 1 min.
4. Remove the spin-column, pour off supernatant and place back into the receiver tube.
5. Continue with point 5. Part II of the protocol (RNA isolation, page 8).

Appendix 2

RNA Purification of plant tissues rich in phenolic compounds and lignins.

NOTE 1 • In the case of plants with high phenolic compounds level, lignins accumulation or problems with downstream reactions (PCR/ RT-qPCR), it is recommended to exchange Lyse ALL buffer with Lyse Buffer PVP cat. no E0291-01. Lyse Buffer PVP contains polyvinylpyrrolidone (PVP) actively reducing plant PCR inhibitors and increasing the efficiency of RNA extraction.

NOTE 2 • The protocol is modified by buffer Lyse ALL exchange to Lyse Buffer PVP followed by samples incubation on ice (step 3).

NOTE 3 • Add either 10 µl β-mercaptoethanol (β-ME) or 10 µl [1 M] DTT per 1 ml Lyse Buffer PVP and RL before use.

NOTE 4 • Lyse Buffer PVP is not supplied with this kit, but is available as a separate product (Cat. no. E0291-01).

1. Choose homogenization method:

   a) Grind plant tissue under liquid nitrogen to a fine powder using previously cooled mortar and pestle. Place sample material (max. 100 mg) in RNase_free, cooled 2 ml Eppendorf tube. Add 200 µl Lyse Buffer PVP and 100 µl **RL** buffer to a plant tissue powder. Mix thoroughly by vigorous vortexing.

   - *Do not use more than 100 mg plant tissues.*
   - *To obtain high yield of RNA a tissue fragment should be thoroughly grinded to a fine powder.*
   - *Frozen plant tissue should not be allowed to thaw during handling.*
   - *Make sure that Lyse Buffer PVP and RL contain β-ME or DTT.*

   b) Place the weighed plant tissue (fresh or frozen) in a suitably sized vessel for homogenizer. Add 200 µl Lyse Buffer PVP and 100 µl **RL** buffer and homogenize using conventional rotor-stator homogenizer until the sample is homogeneous.

   - *If using mortar and pestle, do not use more than 100 mg plant tissues. If using rotor-stator homogenizer use up to 10 times less plant tissues.*
   - *We recommend homogenization with rotor-stator homogenizer for maximum total RNA yields.*

2. Centrifuge sample for 4 min at maximum speed and transfer the supernatant into the new, RNase-free, Eppendorf tube.
3. Incubate sample on ice for 10 min.
4. Continue with step 4 page 7of standard Plant protocol.

Quality Control of Isolated RNA

Quality of isolated total RNA is routinely assessed

— **for purity:** By spectrophotometric measurement

Criteria for high quality RNA:

- A260/A280 ratio 1.8–2.1 (high quality range)
- RNA concentration: A260 = 1 ≈ 40 ng / µl x sample dilution factor

— **for physical integrity:** By agarose gel electrophoresis (1–1.5% [w/v]) — quick check.

Criteria for high quality RNA:

- Distinctly visible, prominent and sharp 28S and 18S rRNA bands, no visible band retardation (indicative for RNase action),
- 28S/18S rRNA band intensity ratio is ≥ 2:1,
- the "smear" appearing among the prominent 28S and 18S rRNA bands represents mRNAs of various sizes and spans the region between both prominently visible bands (the "smear" should not concentrate in the small-sized-RNA region),
- no enrichment of small or very small RNAs is observed (<100 nt), which would be indicative for RNA degradation,
- no visible band appears at ~20 kb (indicative for contamination by genomic DNA),
- total RNA appears in distinct, but tissue-specific patterns.

NOTE 1 · ssRNA Migration Behaviour. 28S rRNA and 18S rRNA are single-stranded molecules, and, due to their biological function, form extensive, stable secondary structures. Both rRNAs will therefore migrate faster through a non-denaturing agarose gel, as compared to a dsDNA molecular weight standard. A direct size comparison of rRNAs to dsDNA marker is thus not possible. However, both ribosomal RNA bands are easily and unambiguously identified due to their prominent intensity and appearance. In contrast, precise RNA size comparisons would require much more work-intense denaturing polyacrylamide gel electrophoresis. For the sole application within a mere routine quality control, the extra workload for preparing and running PAGE gel electrophoresis is, in our eyes, not justified. PAGE gel electrophoresis would add no surplus of relevant information in this application.

NOTE 2 · 28S/18S rRNA Band Intensity Ratio > 2:1. In living cells, 28S rRNA and 18S rRNA, respectively, are always present in a 1:1 stochiometric ratio. Since 28S rRNA is more than double the size of 18S rRNA (for Homo sapiens: 28S rRNA ~5000 nt, 18S rRNA ~1900 nt; similar values apply throughout the animal kingdom): The gel band representing 28S rRNA is supposed to appear with more than double of the intensity as compared to its 18S rRNA counterpart. Thus, a 28S / 18S rRNA ratio of <2 and / or visible band retardation are indicative for RNA degradation.

Safety Information

Lyse ALL

Warning

H319 Causes serious eye irritation.
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.
P337+**P313** If eye irritation persists: Get medical advice/ attention.

DRP

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.
EUH032 Contact with acids liberates very toxic gas.

Wash RB1

Warning

H226 Flammable liquid and vapour.
H302 Harmful if swallowed.
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.
P301+**P312** If swallowed: call a poison center/ doctor/... if you feel unwell.
P403+**P235** Store in a well-ventilated place. Keep cool.
EUH032 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.

GeneMATRIX is synthetic, new generation DNA- and RNA-binding membrane, selectively binding nucleic acids to composite silica structures.

Novel binding and washing buffers are developed to take full advantage of GeneMATRIX capacity, yielding biologically active, high-quality nucleic acids. Matrix is conveniently pre-packed in ready-to-use spin-format. Unique chemical composition of the matrixes along with optimized construction of spin-columns improve the quality of final DNA or RNA preparation. To speed up and simplify isolation procedure, the key buffers are colour coded, which allows monitoring of complete solution mixing and makes purification procedure more reproducible.

As a result, we offer kits, containing matrixes and buffers that guarantee rapid, convenient, safe and efficient isolation of ultrapure nucleic acids. Such DNA or RNA can be directly used in subsequent molecular biology applications, such as: restriction digestion, dephosphorylation, kinasing, ligation, protein-DNA interaction studies, sequencing, blotting, in vitro translation, cDNA synthesis, hybrydization among others. Additional advantage is reproducibility of matrix performance, as component preparation is carried at Eurx Ltd.

GeneMATRIX DNA/RNA/Protein Purification Kit is designed for rapid purification of genomic DNA, total RNA and total protein simultaneously from a single biological sample from a wide variety of bacterial physiological groups, animal tissue, plant and from a wide variety of yeast strains.

Samples are first disrupted, homogenized and lysed in the presence of lysis and denaturing buffers, which inactivates DNases and RNases as well as proteases. In the next stage, DNA binding spin columns reduce viscosity of the lysate and bind DNA fragments. Then sample is applied to a RNA binding spin column where all RNA molecules are adsorbed to the matrix and contaminants are efficiently washed away. High-quality RNA is then eluted in RNase-free water.

Typical yields are up to 100 µg total RNA longer than 200 bases. Proteins are precipitated from the flow-through of RNA binding spin column and are pelleted by centrifugation. The kit includes buffer PLB which is compatible with SDS-PAGE for dissolving the protein pellet. This kit is ideal for researchers who are interested in studying the genome, proteome and transcriptome of a single sample.

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