MB459_IFU_EN_V2401

NZY DNA/RNA/Protein Isolation Kit

Catalogue number
MB45901

Presentation
50 columns

Description

The NZY DNA/RNA/Protein Isolation Kit overcomes challenges in simultaneous DNA, RNA, and protein isolation from small and diverse samples. These samples may include biopsies, tumours, tissues, and transgenic organisms. This kit does not require splitting the sample before extraction, thus providing DNA, RNA, and protein from the exact same portion. DNA and RNA are separately eluted with low salt buffer and water, respectively. All isolated products are suitable for common downstream applications. The NZY DNA/RNA/Protein Isolation method ensures the preservation of DNA, RNA, and protein integrity. Cells are lysed in a chaotropic ion-rich solution, inhibiting enzymes like DNases, RNases, proteases, and phosphatases, and simultaneously allowing DNA and RNA to bind to the silica membrane. This process negates the need for costly proteinase inhibitors. After two specialized washing steps, DNA is selectively eluted using a low salt buffer, without compromising RNA quality or quantity. Any residual DNA is removed by an rDNase solution applied directly to the silica membrane. After the washing steps, RNA is eluted using RNase-free water. Protein is isolated from the initial column flowthrough and precipitated with a protein precipitator buffer (Buffer NPP). The protein pellet is then dissolved in protein solving buffer (Buffer NPS) containing the odourless reducing agent TCEP, ready for SDS-PAGE analysis. Note that this kit is not suitable for the isolation of native proteins.

DNA, RNA, and protein extraction can be performed at room temperature, but due to the sensitivity of RNA to trace RNase contamination, the RNA eluates require careful handling. DNA should be stored at 4 °C for short-term and -20 °C for long-term storage. To maintain RNA stability, store at -20 °C for short-term or -70 °C for long-term. Protein dissolved in protein solving buffer (Buffer NPS) is relatively stable.

Shipping & Storage Conditions

This product is shipped at room temperature.

Upon arrival, store the lyophilized rDNase and the Reducing Agent TCEP at 4 °C; both are stable for up to a year. All other kit components can be stored at room temperature (15–25 °C) and are stable till the expiry date if stored as specified. Be aware that storing at lower temperatures could cause salt precipitation.

Components

COMPONENT	MB45901 (50 COLUMNS)
Buffer NDRPL	25 mL
Buffer NDW (concentrate)	12 mL
Buffer NDE	12 mL
Buffer NDRPW1	15 mL
Buffer NDRPW2 (concentrate)	12 mL
Buffer NPP	45 mL
Buffer NPS (without reducing agent)	7,5 mL
Reducing Agent TCEP	107 mg
RNase-free Water	13 mL
Digestion buffer	7 mL
rDNAse (lyophilized)	1 vial
NZYSpin Homogenization Filter Columns (violet rings)	50
NZYSpin DRP Columns (blue rings)	50
Collection tubes (1.5 mL)	100
Collection tubes (2 mL)	150

Reagents, Materials and Equipment Required but Not Provided

- 50%, 70% and 96-100% ethanol
- 1,5 mL microcentrifuge tubes and disposable tips
- Centrifuge for 1,5 mL microcentrifuge tubes
- Vortex mixer
- Disruption and homogenization equipment's

Specifications

The main specifications of NZY DNA/RNA/Protein Isolation Kit are presented in the following Table 1.

PARAMETER	RNA	DNA	PROTEIN
Sample material	< 8 x 10^6 Cultured Cells < 35 mg Human / Animal Tissue < 110 mg Plant Tissue		
Fragment size	> 200 b	< 30 kbp	15–250 kDa
Typical yield	< 70 µg	< 6 µg	< 1200 µg
A260/A280	1.9–2.1	1.7–1.9	-
Typical RIN*	> 9	-	-
Elution volume	40–120 µL	40–120 µL	100 µL
Preparation time	30 min/6 preps	45 min/6 preps	35 min/6 preps
Binding capacity	200 µg	10 µg	-

* The integrity of RNA strongly depends on the quality of the sample. RNA isolated from fresh and high-quality samples is expected to have a RIN value > 9.

Total DNA: The isolated DNA is of high molecular weight and suitable for PCR.

Total RNA: The RNA purified using this kit has a typical A_{260}/A_{280} ratio above 1.9 and is ready for RT-PCR, primer extension, NGS, and RNase protection assays. The integrity of RNA generally exceeds a RIN of 9.0 but depends on the sample quality.

Total Protein: The protein obtained is ready for SDS-PAGE, Western blot analysis, and quantification. The kit has successfully analysed proteins ranging from 17 kDa to 250 kDa, glycoproteins, membrane proteins, lipoproteins, phosphorylated proteins, and structural proteins. Protein yield depends on sample type, amount, quality, homogenization efficiency, and quantification method. The kit assumes complete processing of the sample. However, in many cases, precipitation of only a portion of the column flow-through is recommended and sufficient for SDS-PAGE and Western blot analysis.

Columns type: silica membrane technology

Elution Volume: 40-120 µL(RNA); 40-120 µL(DNA); 100 µL(Protein)

Processing of Starting Samples

RNA remains vulnerable to degradation until samples are either flash-frozen or disrupted in the presence of RNase-inhibiting or denaturing agents. It is, therefore, crucial to flash freeze samples in liquid nitrogen immediately and store them at -70 °C or process them promptly. Once disrupted in Lysis Buffer NDRPL, samples can be stored at -70 °C for up to a year, +4 °C for up to 24 hours, or at room temperature for several hours. Frozen samples retain stability for up to six months. Prior to RNA isolation, thaw frozen samples in Buffer NDRPL slowly. During preparation, ensure that gloves are always worn and changed frequently for optimal safety and sterility.

To process **Cultured Animal Cells**, collect them by centrifugation and lyse directly using Buffer NDRPL, following the standard protocol (see the section below). For lysis of adherent cells in a culture dish, carefully aspirate the cell-culture medium and immediately introduce Lysis Buffer NDRPL. It is essential to remove all the cell culture media to maximize lysis buffer effectiveness. To trypsinize adherent cells, aspirate the cell culture media, wash the cells with an equivalent volume of PBS, and add 0.1–0.3 % trypsin in PBS. After an appropriate incubation period for cell detachment, add medium, transfer the cells to an appropriate tube, and centrifuge at 300 x g for 5 minutes. Remove the supernatant and add Lysis Buffer NDRPL to the cell pellet.

Solid samples, such as **Human/Animal and Plant Tissues**, require mechanical disruption in addition to lysis for optimal RNA extraction. Depending on the disruption method used, you might need to reduce the sample's viscosity further. This ensures that all RNA is released from the cells and the sample's viscosity is lowered via homogenization. Grinding with a pestle and mortar, done in the presence of liquid nitrogen, is the most common technique for animal tissue disruption. The tissue should then be homogenized with an appropriate device or by multiple passes through a 0.9 mm syringe needle. Undisrupted animal tissue should only be thawed in the presence of Buffer NDRPL during simultaneous mechanical disruption, such as rotor-stator homogenization. This prevents RNA degradation by RNases prior to extraction. Ensure the rotor tip remains submerged to avoid excessive foaming. If foam is present, degenerate it by centrifuging for 1 min at 400 x g. A suitable-sized homogenizer (5–7 mm diameter rotors) can be used in microcentrifuge tubes.

For **Bacteria and Yeast**, incubation in lysozyme or lyticase/zymolase solutions, respectively, is necessary to digest or weaken the robust cell walls, enabling effective lysis with Buffer NDRPL. For organisms with particularly resistant cell walls, such as certain Gram-positive bacteria, treatment conditions may need optimization. Following lysis, achieve homogenization with the syringe-needle method.

The Table below (**Table 2**) provides an initial guideline for selecting suitable quantities of sample material, precipitation volume, and resolubilization volume. It is crucial to note that the ideal volumes may differ from those outlined in the table, depending on the sample type and downstream application (for instance, Coomassie or silver stain, antibody sensitivity, and the detection system used). Thus, these appropriate volumes should be validated empirically.

Table 2. Recommended buffer volumes to use for appropriate sample amount.

SAMPLE	AMOUNT	COLUMN FLOWTHROUGH TO BE PRECIPITATED	BUFFER NPP FOR PROTEIN PRECIPITATION	BUFFER NPS FOR PROTEIN PELLET SOLUBILIZATION	PROTEIN SAMPLE FOR SDS-PAGE *	PROTEIN SAMPLE FOR WESTERN BLOT
Cultivated cells (e.g., HeLa cells)	10^6	35 µL	35 µL	100 µL	10 µL	1–10 µL
	10^5	350 µL	350 µL	100 µL	10 µL	1–10 µL
	10^4	700 µL	700 µL	20 µL	10 µL	1–10 µL
Animal tissue (e.g., liver)	30 mg	35 µL	35 µL	100 µL	10 µL	1–10 µL
	3 mg	350 µL	350 µL	100 µL	10 µL	1–10 µL
	0,3 mg	700 µL	700 µL	20 µL	10 µL	1–10 µL
Plant tissue (e.g., garden leaf)	100 mg	35 µL	35 µL	100 µL	10 µL	1–10 µL
	10 mg	350 µL	350 µL	100 µL	10 µL	1–10 µL
	1 mg	700 µL	700 µL	20 µL	10 µL	1–10 µL

* Volumes for Coomassie staining. For Silver staining use 1-2 µL.

Standard Protocol

Recommendations before starting

- Ensure the availability of 70% ethanol, which is an additional solution used to adjust binding conditions in the NDRPL-lysate
- Verify the presence of 50% ethanol, required for washing the protein pellet and preparing the Buffer NDW.
- Make sure you have 96–100% ethanol on hand to prepare Wash Buffer NDRPW2.
- The Buffer NDE (DNA elution buffer) does not contain DNase inhibitors (such as EDTA) or substances that could prevent microbial growth due to its composition. Therefore, be vigilant to avoid contaminating the Buffer NDE with DNases or any microbial source.

Procedures before starting

Reagents Preparation

- **Buffer NDW**: Add 48 mL of 50% ethanol to the Buffer NDW concentrate bottle. Keep the bottle tightly closed to prevent ethanol evaporation.
- **rDNAse**: Add 540 µL of RNase-free water to the rDNase vial. Allow it to incubate for 1 minute at room temperature. Gently swirl the vials to fully dissolve the rDNase. Avoid vigorous mixing as rDNase is sensitive to mechanical agitation. Aliquot and store at -15 to -25 °C. The frozen solution is stable for 6 months. Do not freeze/thaw the aliquots more than three times.
- **Buffer NDRPW2**: Add 48 mL of 96–100% ethanol to the Buffer NDRPW2 concentrate bottle. Note the addition of ethanol on the bottle label. Store the Buffer NDRPW2 at room temperature for up to a year, keeping the bottle tightly closed to avoid ethanol evaporation.
- **Buffer NPS** (protein solving buffer) and **Reducing Agent TCEP**: If performing SDS-PAGE under reducing conditions (the most common type), transfer Buffer NPS from one vial to vial of the Reducing Agent TCEP. Mix gently to prevent excessive foaming until the reducing agent is fully dissolved (this process will take several minutes). NPS-TCEP solution is stable for several days at room temperature and for several months at 4 °C. For long-term storage, keep NPS-TCEP solution at -20 °C.

  Consider the following if performing SDS-PAGE under non-reducing conditions: Do not add the Reducing Agent TCEP to Buffer NPS. Do not add ß-mercaptoethanol (or other reducing agent) to Lysis Buffer NDRPL. If preferred reducing agents other than TCEP (e.g., DTT, ß-mercaptoethanol), add appropriate amounts to Buffer NPS. Note the limited stability of DTT compared to TCEP. If NPS -TCEP solution appears cloudy, warm it up to > 25 °C before use until the solution is completely clear (i.e., all precipitate is fully dissolved). NPS-TCEP solution has a half-life of approximately 5 months if stored at 4 °C and approximately 7 months if stored at -20 °C.

Procedure

Prior to beginning the protocol, please ensure that buffers and reagents have been prepared in accordance with the instructions provided above. The following procedures allow to isolate DNA, RNA and Protein using the same starting material. Please adhere to the provided instructions for each extraction process.

- DNA purification: steps 1–8
- RNA purification: steps 1–11
- Protein purification: steps 1–5 and 12–15

DNA & RNA Purification Steps

1. Sample Homogenization

Homogenize up to 30 mg of human/animal tissue or up to 100 mg of plant tissue (see above). Eukaryotic cultured cells (up to 5 x 10^6) are collected via centrifugation and lysed directly with Buffer NDRPL.

2. Sample Lysis

Combine 350 µL Buffer NDRPL and 3.5 µL ß-mercaptoethanol (ß-ME) with the cell pellet or ground tissue. Vortex vigorously.

Note: *DTT or TCEP can replace ß-ME. Ensure the final concentration within the Lysis Buffer NDRPL is 10–20 mM for DTT or TCEP.*

3. Lysate Filtration

To reduce viscosity and clear the lysate, apply the lysate to an NZYSpin Homogenization Filter Column (violet ring) placed in a 2 mL collection tube and centrifuge for 1 minute at 11,000 x *g*.

Note: *If a visible pellet forms in the collection tube, transfer the supernatant without the pellet to a new 2 mL centrifuge tube.*

4. DNA and RNA Binding Conditions

Discard the NZYSpin Homogenization Filter Column (violet ring), add 350 µL of 70% ethanol to the homogenized lysate, and mix. Alternatively, transfer flowthrough to a new 1.5 mL microcentrifuge tube, add 350 µL 70% ethanol and vortex. After adding ethanol, a stringy precipitate might appear. This will not interfere with RNA isolation. Ensure all precipitate is disaggregated by mixing before loading onto the column (step 5).

Note: *Avoid centrifugation at this stage to prevent sedimentation.*

5. DNA and RNA Binding

Load the lysate onto an NZYSpin DRP Column (blue ring) placed in a collection tube and centrifuge for 30 seconds at 11,000 x *g*.

For DNA and RNA isolation, continue with step 6.

For protein isolation, use the flowthrough and continue with step 12.

6. Silica Membrane Washing

Perform a 1st and 2nd wash with 500 µL Buffer NDW, centrifuging for 1 minute at 11,000 x *g* each time and discarding the flowthrough afterwards.

7. Membrane Drying

Place the NZYSpin DRP Column (blue ring) into a 1.5 mL microcentrifuge tube and let it stand open for 3 minutes to evaporate residual ethanol.

8. DNA Elution

Add 100 µL Buffer NDE directly onto the membrane, incubate for 1-5 minutes, and then centrifuge for 1 minute at 11,000 x *g* to elute the DNA. The temperature of the DNA elute solution shall not exceed 30 °C, otherwise, RNA will partly elute with the Buffer NDE. Buffer NDE may stay for 1 minute up to 15 minutes on the column before DNA is eluted. A 1–5 minutes incubation time is recommended.

9. On-Column DNA Digestion (optional)

Prepare rDNase reaction mixture (10 µL reconstituted rDNase in 90 µL Digestion Buffer) and apply 95 µL onto the center of the silica membrane. Incubate at room temperature for 15 minutes.

10. Wash and Dry Silica Membrane

Add 200 µL Buffer NDRPW1, centrifuge for 30 seconds at 11,000 x *g*. Then, perform a 2nd and 3rd wash with 600 µL and 250 µL Buffer NDRPW2, respectively, each time followed by centrifugation.

11. RNA Elution

Elute RNA in 60 µL RNase-free water and centrifuge at 11,000 x *g* for 1 minute.

Protein Purification Steps

12. Protein Precipitation

Into a fresh collection tube (1.5 mL, supplied), add one volume of Buffer NPP (protein precipitator buffer) to the flowthrough from step 5, mix vigorously, and incubate at room temperature for approximately 10 minutes. Check the volume of flowthrough and Buffer NPP in **Table 2** above. Centrifuge at 16,000 x *g* for 5 minutes to precipitate the proteins.

13. Protein Wash

Completely remove the supernatant from the sample. Add 500 µL of 50% ethanol to the pellet.

Note: *that there is no need for mixing or incubation at this step. Centrifuge the sample for 1 minute at 11,000 x g, then remove any remaining supernatant as thoroughly as possible.*

14. Drying Protein Pellet

Allow the precipitate to dry for 5-10 minutes at room temperature. Leave the tube open during this process.

Note: *Larger pellets, like those resulting from a complete precipitation of 700 µL column flow-through from a 30 mg liver sample, may require more time to dry. Be aware that if samples are not fully dried, the residual ethanol content might cause issues when loading the sample onto the gel. However, over-drying doesn't pose a problem for smaller pellets.*

15. Protein sample preparation

To prepare the protein sample, add 20–100 µL of NPS-TCEP solution, ensuring it is clear and not turbid. If necessary, heat the NPS-TCEP solution to more than 25 °C to dissolve any turbidity. See the section above for guidelines for choosing an appropriate amount of NPS-TCEP solution.

Break down any large and visible pellets with a pipette tip to help with protein dissolution. This step is not necessary for smaller or invisible pellets. Incubate the sample for 3 minutes at 95–98 °C to completely dissolve and denature the protein. Allow the sample to cool to room temperature.

Centrifuge for 1 minute at 11,000 *x g* to pellet any remaining insoluble material. Recover the supernatant for further analysis.

Notes:

- *Depending on the sample, there might be no visible pellet or pellets of different sizes and structures. Be careful not to disturb any residual precipitates at this stage. The protein will be in the supernatant. Avoid centrifuging samples at temperatures below 18 °C as SDS may precipitate at this temperature.*
- *At this point, the samples can be stored at -20 °C for several months or at 4 °C for several days. After storage, allow the sample to reach room temperature, mix it, and then briefly centrifuge before taking sample aliquots. Repeatedly denaturing the sample for 3 minutes at 95–98 °C is not necessary. You can withdraw, freeze, and thaw the sample at least three times without affecting sample quality.*
- *Due to the strong denaturing purification method, the protein is precipitated in denatured form with reduced solubility in water. Therefore, it's recommended to resolubilize the protein pellet in NPS -TCEP solution or in traditional Laemmli buffer. The use of Protein Solving Buffer NPS is not mandatory for dissolving protein. Alternatively, precipitated protein can be dissolved in 1% SDS or 8M urea. The protein pellet can also be dissolved in urea/thiourea/CHAPS buffers used for 2-D electrophoresis. However, the overall yield of solubilized protein may be reduced compared to using NPS or NPS-TCEP solution as the dissolving agent.*

Protocol for rDNase digestion in solution (Optional)

The standard on-column rDNase digestion protocol delivers RNA with minimal residual DNA, which remains undetectable in most downstream applications. Nevertheless, some applications may require even less residual DNA. Eliminating DNA to a completely undetectable level is difficult, and the on-column DNA digestion may not suffice for applications that need extremely low residual DNA. This protocol revision is particularly relevant for RT-PCR reactions where primer molecules cannot differentiate between cDNA (from RNA) and genomic DNA contaminants. It is especially crucial when analysing high copy number targets, dealing with very low expression level target genes, or handling relatively small amplicons (< 200 bp). The rDNase included in this NZYtech kit is of high quality, recombinant, and RNase-free, enabling an efficient DNA digestion in solution. The following protocol helps to eliminate even trace DNA contaminants.

1. For DNA Digestion mixture preparation, add 6 µL of Digestion Buffer and 0.6 µL of rDNase to 60 µL of the eluted RNA. Alternatively, mix 100 µL of Digestion Buffer and 10 µL of rDNase and add one-tenth of the volume to the RNA eluate.
2. Incubate the sample for 10 minutes at 37 °C.
3. Repurify the RNA using a suitable RNA clean-up procedure or ethanol precipitation protocol.

   Ethanol Precipitation protocol:

   - Add 0.1 volume of 3 M sodium acetate (pH 5.2) and 2.5 volumes of 96-100% ethanol to one volume of the sample. Mix thoroughly.
   - Incubate for a few minutes to several hours at -20 °C or 4 °C.

   Note: *Longer incubation times are needed for samples with low RNA concentrations. Shorter times are sufficient for samples with high RNA concentrations).*

   - Centrifuge for 10 minutes at maximum speed.
   - Wash the RNA pellet with 70% ethanol.
   - Dry the RNA pellet and re-suspend the RNA in RNase-free water.

Technical Notes

Protein Buffers

The NZY DNA/RNA/Protein Isolation Kit includes a Protein Solving Buffer (Buffer NPS) and a Reducing Agent TCEP. Buffer NPS, similar to the well-known Laemmli buffer in composition and function, can be used interchangeably with it in most cases. However, for larger protein pellets (approximately 1 mm), the use of Buffer NPS is suggested. TCEP serves as a versatile, potent, and odourless reducing agent, exhibiting resistance to air oxidation, unlike common reducers such as DTT and ß-mercaptoethanol. TCEP effectively breaks disulfide bonds, even in the most robust

water-soluble alkyl disulfides, across a broad pH range. Mixing TCEP with Buffer NPS, as instructed, will result in a NPS -TCEP solution with a TCEP concentration of 50 mM, sufficient to efficiently reduce peptide and protein disulfide bonds within minutes (up to a protein concentration of approximately 1 µg/µL).

Elution procedures

DNA elution is conducted under selective conditions to ensure that only DNA is released while RNA remains bound to the membrane. The fine-tuning of DNA Wash solution and DNA Elute buffer allows for this specificity. The DNA elution volume should only be moderately adjusted, within the 60–150 µL range. The DNA Elute solution's temperature must not exceed 30 °C to prevent RNA co-elution. DNA Elute can remain on the column for 1 to 15 minutes, although 1–5 minutes is advisable. The eluted DNA is ready for immediate downstream applications without further purification.

The RNA elution method and water elution volume can be tailored to the specific downstream application. Beyond the standard method detailed in the individual protocols (recovery rate approximately 70–90%), several modifications are possible. For **high yield**, perform two elution steps using the volume specified in the individual protocol to achieve approximately 90–100% of bound nucleic acid elution. For both **high yield and high concentration**, elute with the standard volume and then re-elute by applying the eluate once more onto the column. Immediately place the eluted RNA on ice and keep it chilled for optimal stability and to prevent degradation by ubiquitous RNases (found in general labware, fingerprints, dust). For short-term storage, freeze at -20 °C; for long-term storage, freeze at -70 °C.

Quality control assay

All components of NZY DNA/RNA/Protein Isolation kit are tested following the isolation protocol described above. The purification system must isolate 50-70 µg of Total RNA, 3-6 µg of Total DNA and 1000-1200 µg of Total Protein, depending on the source of the tested samples.