Presto™ DNA/RNA/Protein Kit Quick Protocol

For research use only

Catalogue Number

DRP004, DRP050, DRP100

Instruction Manual Download

When using this product for the first time, or if you are unfamiliar with the procedure, please scan the QR code and download the complete instruction manual.

Instruction Manual Download

IMPORTANT BEFORE USE!

1. Add absolute ethanol (see the bottle label for volume) to RPE Buffer and Wash Buffer. Mix by shaking for a few seconds. Check the box on the bottle. Close the bottle tightly after each use to avoid ethanol evaporation.

2. Prepare Phosphate Buffered Saline (PBS, pH7.2) for adherent cultured cells.

3. Yield and quality of DNA/RNA will be higher when fresh samples or samples which have been flash frozen and stored at -70ºC are used. DNA/RNA in samples which are repeatedly frozen and thawed may be degraded.

Additional Requirements

absolute ethanol, ß-mercaptoethanol, acetone, for cell samples: phosphate-buffered saline (PBS), 0.10-0.25% Trypsin, for tissue samples: TissueLyser or mortar and pestle, 20-G needle syringe

NOTE: Dithiothreitol (DTT) can be used as an alternative reducing agent in DR Buffer instead of ß-mercaptoethanol. Immediately prior to use, add 40 mM DTT per reaction to DR Buffer. For example, add 20 µl of 2 M DTT in RNase-free Water to 1 ml of DR Buffer then mix well.

1. Sample Preparation

Adherent Cultured Animal Cells

A. Cell lysis in a culture dish

Aspirate the culture medium completely. Add **400 µl of DR Buffer and 4 µl of ß-mercaptoethanol** immediately to the culture dish (up to 5 x10^6 cells). Incubate at room temperature for 5 minutes then transfer the cell lysate to a 1.5 ml microcentrifuge tube.

B. Trypsinize cell prior to cell lysis

Remove the culture medium and wash cells in PBS. Aspirate PBS and add 0.10-0.25% Trypsin in PBS. Once cells have detached, add the medium and transfer to a 15 ml centrifuge tube. Proceed with Suspension Cultured Animal Cells.

Suspension Cultured Animal Cells

Transfer cells (up to 5 x 10^6) to a 1.5 ml microcentrifuge tube or 15 ml centrifuge tube then centrifuge for 5 minutes at 300 x g. Remove the supernatant completely then add **400 µl of DR Buffer and 4 µl of ß-mercaptoethanol** to resuspend cells by pipetting or vortex. Proceed with DNA/RNA Separation.

Animal Tissue

Excise 10-25 mg of tissue directly from the animal or remove the tissue sample from storage. Do not use more than 25 mg of tissue per reaction. Homogenize tissue samples using one of the following methods: A. Transfer the tissue to a 2 ml centrifuge tube containing ceramic beads or stainless steel beads, add **400 µl of DR Buffer** and **4 µl of ß-mercaptoethanol** to the tissue sample and then homogenize the sample with a TissueLyser, Disruptor Genie or similar. B. Freeze the tissue in liquid nitrogen then grind the tissue thoroughly with a mortar and pestle. Transfer the tissue powder to a 1.5 ml microcentrifuge tube (do not allow the tissue to thaw) and add **400 µl of DR Buffer** and **4 µl of ß-mercaptoethanol**. Shear the tissue by passing the lysate through a 20-G needle syringe 10 times. Proceed with DNA/RNA Separation.

Biological Fluids

Add **300 µl of DR Buffer** and **3 µl of ß-mercaptoethanol** to 100 µl of liquid sample and mix well by pipetting or vortex. Proceed with DNA/RNA Separation.

Human Whole Blood

Collect fresh human blood in anticoagulant-treated collection tubes. Transfer 500 µl of blood to a sterile 15 ml centrifuge tube. Add **1.5 ml of RBC Lysis Buffer (3 volumes)** and mix by inversion. Incubate the tube on ice for 10 minutes (vortex twice briefly during incubation). Centrifuge at 3,000 x g for 5 minutes then remove the supernatant completely. Add **400 µl of DR Buffer** and **4 µl of ß-mercaptoethanol** to resuspend leukocyte pellet by pipetting or vortex. Proceed with DNA/RNA Separation.

2. DNA/RNA Separation

Incubate the sample lysate at room temperature for 5 minutes then centrifuge at 12-16,000 x g for 2 minutes. Place a **GD Column** in a 2 ml Collection Tube then transfer the supernatant to the **GD Column**. Centrifuge at 14-16,000 x g for 1 minute. NOTE: If the lysate mixture could not flow past the GD Column membrane following centrifugation, increase the centrifuge time until it passes completely. Save the flow-through in the 2 ml Collection Tube for RNA Purification. Place the **GD Column** in a new 2 ml Collection Tube and store at room temperature (15-25ºC) or 4ºC for DNA Purification. Do not store the GD Column for extended periods. Do not freeze the GD Column. At this time, preheat the required Elution Buffer (200 µl per sample) to 60ºC (for DNA elution).

RNA Purification

3. RNA Binding

Add a **0.8 volume of absolute ethanol** to the flow-through in the 2 ml Collection Tube (e.g. add 320 µl of absolute ethanol to 400 µl of flow-through) and mix well by pipetting. Transfer the sample to the **RB Column** in a 2 ml Collection Tube. Centrifuge at 14-16,000 x g for 1 minute. Place the **RB Column** in a new 2 ml Collection Tube and save the flow-through for protein purification. NOTE: If DNA-free RNA is required, perform optional In Column DNase I Digestion below.

Optional In Column DNase I Digestion

Add 400 µl of RPE Buffer (make sure ethanol was added) to the RB Column then centrifuge at 14-16,000 x g for 30 seconds. Discard the flow-through and place the RB Column back in the 2 ml Collection Tube. Prepare DNase I solution in a 1.5 ml microcentrifuge tube (RNase-free) as follows:

DNase I	5 µl (2 U/µl)
DNase I Reaction Buffer	45 µl
Total Volume	50 µl

RNase-Free DNase I set can be purchased directly from Geneaid (cat. # DNS050/100/300). Standard DNase buffers are incompatible with this in-column DNase digestion, which will affect RNA integrity and reduce yield.

Mix DNase I solution by pipetting gently (DO NOT vortex). Add DNase I solution (50 µl) into the CENTER of the RB column matrix. Incubate the column for 15 minutes at room temperature (20-30ºC) then proceed with RNA Wash below.

4. RNA Wash

Add **400 µl of RW1 Buffer** into the **RB Column** and centrifuge at 14-16,000 x g for 30 seconds. Discard the flow-through and place the **RB Column** back in the 2 ml Collection Tube. Add **600 µl of RPE Buffer** (make sure ethanol was added) into the **RB Column**. Centrifuge at 14-16,000 x g for 30 seconds. Discard the flow-through and place the **RB Column** back in the 2 ml Collection Tube. Add **600 µl of RPE Buffer** (make sure ethanol was added) into the **RB Column**. Centrifuge at 14-16,000 x g for 30 seconds. Discard the flow-through and place the **RB Column** back in the 2 ml Collection Tube. Centrifuge at 14-16,000 x g for 3 minutes to dry the column matrix.

5. RNA Elution

Place the **RB Column** in a clean 1.5 ml microcentrifuge tube (RNase-free). Add **25-50 µl of RNase-free Water** into the CENTER of the column matrix. Let stand for at least 1 minute to ensure the RNase-free Water is absorbed by the matrix. Centrifuge at 14-16,000 x g for 1 minute to elute the purified RNA.

6. Protein Precipitation

Add **4 volumes of ice-cold acetone** to the flow-through from step 3 in a 15 ml centrifuge tube (example: add 2.8 ml of ice-cold acetone to 700 µl flow-through). Incubate on ice or at -20ºC for 30 minutes. At this time, perform DNA Purification below. Centrifuge at 14-16,000 x g for 10 minutes then discard the supernatant.

7. Protein Resuspension

Add **100 µl of ice-cold 70% ethanol** to wash the protein pellet. Discard the supernatant then air-dry the protein pellet at room temperature. NOTE: DO NOT overdry the protein pellet as it may results in difficult resuspension. Add up to **100 µl of DV buffer (8M urea)** or a buffer compatible with downstream application to dissolve the protein pellet.

8. SDS-PAGE Analysis

Add **2 µl of Protein Loading Dye and 0.5 µl of 2M DTT (optional)** into a clean microcentrifuge tube. Add **8 µl of the protein sample** then mix well by pipetting. Incubate protein sample at 100ºC for 3-5 minutes. Centrifuge the protein sample briefly then directly load to SDS-PAGE.

9. DNA Wash

Add **400 µl of W1 Buffer** to the **GD Column**. Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through. Place the **GD Column** back in the 2 ml Collection Tube. Add **600 µl of Wash Buffer** (make sure absolute ethanol was added) to the **GD Column**. Centrifuge at 14-16,000 x g for 30 seconds then discard the flow-through. Place the **GD Column** back in the 2 ml Collection Tube. Centrifuge again for 3 minutes at 14-16,000 x g to dry the column matrix.

NOTE: Additional centrifugation at 14-16,000 x g for 5 minutes or incubation at 60ºC for 5 minutes will completely dry the GD Column to avoid any residual ethanol carryover and ensure the most effective downstream applications.

10. DNA Elution

Standard elution volume is 100 µl. If less sample is to be used, reduce the elution volume (30-50 µl) to increase DNA concentration. If higher DNA yield is required, repeat the DNA Elution step to increase DNA recovery and the total elution volume to approximately 200 µl.

Transfer the dried **GD Column** to a clean 1.5 ml microcentrifuge tube. Add **100 µl of pre-heated Elution Buffer**[1], TE Buffer[2] or water[3] into the CENTER of the column matrix. Let stand for at least 3 minutes to allow Elution Buffer, TE Buffer or water to be completely absorbed. Centrifuge at 14-16,000 x g for 30 seconds to elute the purified DNA.

[1]Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25ºC) is added into the center of the GD Column matrix and is completely absorbed.

[2]Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. Ensure that TE is added into the center of the GD Column matrix and is completely absorbed.

[3]If using water for elution, ensure the water pH is between 7.0 and 8.5. ddH2O should be fresh as ambient CO2 can quickly cause acidification. Ensure that water is added into the center of the GD Column matrix and is completely absorbed. DNA Eluted in water should be stored at -20ºC to avoid degradation.

Presto™ DNA/RNA/Protein Extraction Kit Functional Test Data

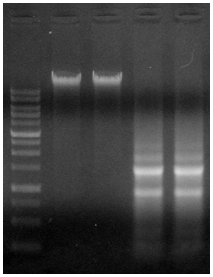

Figure 1. Genomic DNA and Total RNA from 1.5 x 10^6 HeLa cells was extracted using the Presto™ DNA/RNA/Protein Extraction Kit. 10 µl aliquots from a 200 µl eluate of purified genomic DNA and 10 µl aliquots from a 50 µl eluate of purified total RNA were analyzed by electrophoresis on a 1% agarose gel.
1-2 = DNA from 1.5 x 10^6 HeLa cells
3-4 = RNA from 1.5 x 10^6 HeLa cells
M = Geneaid 1 Kb DNA Ladder

Sample	µg/ml	260/280	260/230	Yield
1. DNA	42.7	1.88	2.19	8.54 µg
2. DNA	46.7	1.88	2.18	9.34 µg
3. RNA	417.5	2.09	2.03	20.90 µg
4. RNA	429.9	2.09	2.04	21.50 µg

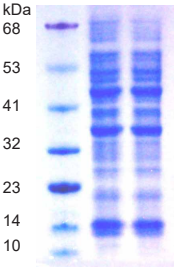

Figure 2. Protein was extracted from 1.5 x 10^6 HeLa Cells using the Presto™ DNA/RNA/Protein Extraction Kit. 20 µl of aliquots from a 200 µl eluate of purified protein were analyzed on a NuPAGE4-12% Bis-Tris gel and stained with coomassie blue.

Kit Components

Component	DRP004	DRP050	DRP100
RBC Lysis Buffer	10 ml	100 ml	200 ml
DR Buffer	2 ml	30 ml	60 ml
RW1 Buffer	2 ml	30 ml	50 ml
RPE Buffer[1] (Add Ethanol)	1 ml (4 ml)	25 ml (100 ml)	50 ml (200 ml)
W1 Buffer	2 ml	45 ml	45 ml
Wash Buffer[1] (Add Ethanol)	1 ml (4 ml)	12.5 ml (50 ml)	25 ml (100 ml)
RNase-free Water	1 ml	6 ml	6 ml
Elution Buffer	1 ml	30 ml	30 ml
DV Buffer (8M Urea)	500 µl	6 ml	12 ml
Protein Loading Dye	60 µl	2 ml	2 ml
RB Column	4	50	100
GD Column	4	50	100
2 ml Collection Tube	16	200	400

[1]Add absolute ethanol (see the bottle label for volume) to RPE Buffer and Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Steps to prevent RNase contamination

1. Always wear a lab coat, disposable gloves, protective goggles and (anti-fog) procedure mask.
2. Plasticware and automatic pipettes should be sterile (RNase-free) and used only for RNA procedures.
3. Non-disposable glassware or plasticware should also be sterile (RNase-free).

Storage: dry at room temperature (15-25ºC)