DUCKY BIO

User Guide

Residual DNA Sample Preparation Kit

Cat. No. S24020201 100 Reactions

Store the kit at 20 to 25°C.

Note: For safety and biohazard guidelines, see the "Safety" appendix in the Residual DNA Sample Preparation Kit *User Guide*. Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Version 01

Ducky Biotechnology Co., Ltd.

This product is for research purposes only. Not for use in diagnostic procedures.

The information in this guide is subject to change without notice.

DISCLAIMER: TO THE EXTENT ALLOWED BY LAW, DUCKY BIOTECHNOLOGY INC. AND/OR ITS AFFILIATE(S) WILL NOT BE LIABLE FOR SPECIAL, INCIDENTAL, INDIRECT, PUNITIVE, MULTIPLE, OR CONSEQUENTIAL DAMAGES IN CONNECTION WITH OR ARISING FROM THIS DOCUMENT, INCLUDING YOUR USE OF IT.

IMPORTANT：Before using this product, read and understand the information in the “Safety” appendix in this document.

Important Licensing Information: These products may be covered by one or more Limited Use Label Licenses. By use of these products, you accept the terms and conditions of all applicable Limited Use Label Licenses.

TRADEMARKS: All trademarks are the property of Ducky Biotechnology and its subsidiaries unless otherwise specified.

1. Product description

The Residual DNA Sample Preparation Kit uses chemical lysis and magnetic beads to extract DNA from diverse sample types, including samples that contain high protein and low DNA concentration. The kit extracts residual genomic DNA from products that are produced in cell lines such as CHO, E. coli, E1A, HEK293, Vero, NS0 and Baculovirus. For quantification of residual DNA, we recommend using the resDNASEQ Residual DNA Quantitation kit as described in the resDNASEQ Residual DNA Quantitation kit User Guide.

To ensure accurate quantitative results, each sample in triplicate and perform a single PCR reaction for each extraction.

2. Contents and storage

The kits contain reagents sufficient for 100 extractions.

Contents	Amount	Storage
Binding Solution	60mL	15℃-25℃（room temperature）
Wash Solution	100mL	
Elution Buffer	10mL	
Proteinase K	2×1.5mL	2℃-25℃
Magnetic Beads	1mL	
User Guide	1	/

* Do not freeze any component.

3. Required materials not supplied for manual protocols

Magnetic stand; Block heater for use with 2-mL tubes; Benchtop microcentrifuge for 1.5-mL and 2-mL tubes; Vortex; Disposable gloves; Aerosol-resistant micropipette tips; Pipettors(1000μL, 200μL, 20μL, 10μL); Nonstick, RNase-free Microfuge Tubes, 1.5-mL;

4. Good laboratory practices

4.1. Good laboratory practices for PCR and RT-PCR

To get a reliable result, perform the workflow setup under DNA-free conditions. This includes:

- Prepare and pipette all solutions with nuclease-free, DNA-free equipment and consumables.
- UV-treat or the laminar flow hood prior to pipetting.

- Use sterile single-use gloves and freshly laundered laboratory coats. Change gloves if you suspect that they are contaminated.
- Close vials immediately after pipetting. Avoid splashing or spraying samples.
- Spatial segregation of the sequential workflow steps.

Rooms	Workflow Step
Sample preparation room	Extraction and purification of test samples,including preparation of recovery control sample.
Master mix preparation room	Master mix preparation and pipetting of PCR Negative Control to the NTC wells.
PCR room for setup and amplification run	Dilution and pipetting of samples and PCR Positive Control to the PCR plate. Running the PCR Instrument.

Note：Do not use disinfectants during Sample preparation experiments so as not to affect the results.

4.2. Avoiding false positives due to cross-contamination

To avoid false positives due to cross-contamination:

- Use a positive-displacement pipettor or aerosol-resistant barrier pipette tips.
- Prepare and close all negative control and unknown sample tubes before pipetting the positive control.
- Do not open tubes after amplification.
- Use different sets of pipettors when pipetting negative control, unknown, and positive control samples.
- In the process of preparing the sample, dispense tubes in sequence from left to right the: negative controls, unknown samples and extraction/recovery control (ERC), and positive controls.

5. Manual Sample Preparation

5.1. Preparation for Preparing DNA

In combination with this kit, a manual sample preparation with the Magnetic Stand (Cat. No. M24040801) is recommended.

Note that due to different types of matrices (i.e. high protein amounts or very high DNA amount), the test samples should be appropriately diluted before running the sample preparation. For this purpose, use the Dilution Buffer provided in resDNASEQ Residual DNA Quantitation kitthis kit.

The correct pH of the sample solutions (pH 7.5) is very important for good DNA recovery.

Test samples from the early purification process often contain levels of DNA/RNA that are above the highest point of the assay standard curve. You must dilute these samples (from 1:100 up to 1:10,000) before sample preparation.

- Dilute test samples before DNA extraction with dilution buffer.

Note：Diluting samples in water or TE reduces extraction efficiency.

- Use the dilution buffer as the negative extraction control instead of water.
- Alternatively, dilute extracted DNA with elution buffer before running the PCR reaction.

Triplicate extractions are required for post-PCR analysis calculation of mean quantity, standard deviation, and coefficient of variation.
In addition to test samples, we recommend triplicate extractions for the negative control and the extraction/recovery control (ERC).
Perform a single PCR reaction for each extraction.

5.2. Procedure for Preparing DNA from 100 µL Test Sample

Figure 1 describes the protocol for preparing DNA from the 100 µl test sample. Use Workflow A for each test sample to be analyzed.
Run once per sample preparation:

- Workflow A: to prepare the Workflow Test Sample.
- Workflow B: to prepare the Workflow Negative Control.
- Workflow C: to prepare the Workflow Positive Control.
- Optional: Workflow D to prepare the Recovery Control.

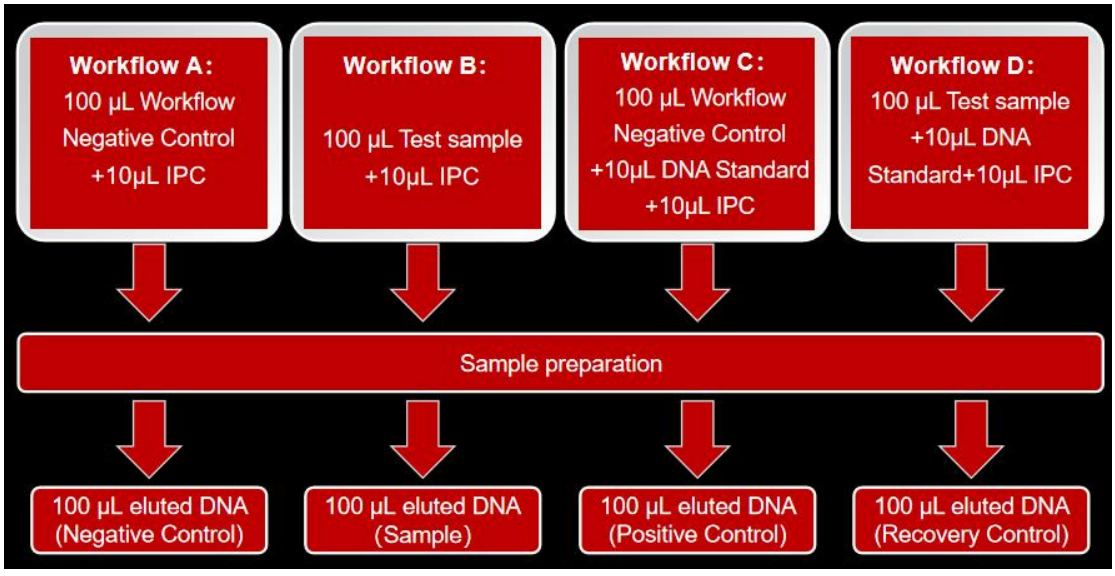

Figure 1 Experimental overview of the Sample preparation.

Add a volume of positive control standard dilution to each test sample so that the total DNA/RNA amount is 2-10 times the amount of DNA/RNA measured in the test sample without the addition of the DNA/RNA control.
IPC Sample (Internal Positive Control) is offered in the Residual DNA Quantitation kit.
The following protocol describes the sample preparation for different matrices derived from samples, in order to obtain a DNA sample as PCR template in good yield.

Instead of the Workflow Negative Control you can also use the Dilution Buffer.

Note: To calculate the efficiency of DNA recovery and quantification from the test samples, subtract the amount of DNA measured in the sample without the addition of DNA control from the amount of DNA/RNA measured in the ERC sample.

6. Manual protocol for DNA extraction

6.1. Before You Begin

1. Set a block heater to 37°C. If available, set a second block heater to 80°C.
2. Balance all reagents and samples to room temperature for accurate pipetting.
3. Label 1.5-mL/2-mL Safe-Lock tubes:

- 3 for workflow A
- 3 for workflow B
- 3 for workflow C
- 3 for workflow D

4. Cool samples to room temperature.
5. Remove the magnetic plate from the magnetic stand, put the tubes on the magnetic stand.

6.2. Bind the DNA/RNA

1. Vortex the Magnetic Beads thoroughly to resuspend the Beads.

Note: The appearance of the mixture should be homogeneous, especially the bottom sediment.

2. Add 10 µL of the Magnetic Beads, 10 µL of Proteinase K and 10µL IPC（using the Internal Positive Control offered by resDNASEQ Residual DNA Quantitation kit, Red cap）to each tube.
3. Add 600 µL Binding Solution to each tube.
4. Add 100µL workflow A, 100 µL workflow B, 110µL workflow C, and 110µL workflow D into each tube, then close the cap.
5. Vortex each tube thoroughly, then centrifuge Briefly.
6. Incubate at 37℃for 2 minutes.

If only one block heater is available, after this incubation step is complete, reset

the block heater to 80℃ for the elution step.

7. PPlace the tubes in the magnetic stand, insert the plate, then let the tubes stand until the solution is clear. The magnetic stand from Ducky Bio is recommended.

8. Open the cap, without disturbing the Magnetic Beads, then remove the supernatant by pipette or by vacuum pump.

Note：In the process of removing the supernatant, do not take away the magnetic beads.

Note：Be careful not to have any liquid residue on the cap and wall of the tube as well as the bottom of the tube.

6.3. Wash the DNA/RNA

1. Remove the magnetic plate from the magnetic stand, then add 20μL of Proteinase K and 1000 μL of Wash Solution to the tubes, then close the cap.

2. Vortex the tubes for 10 seconds at room temperature at 900 rpm.

3. Centrifuge the tubes in a microcentrifuge at top speed (>3,000 × g) for a maximum of 5 seconds.

Do not centrifuge for >5 seconds.

4. Insert the magnetic plate into the magnetic stand, then let the tubes stand for 2 minute or until the solution is clear.

5. Open the cap, without disturbing the Magnetic Beads, then remove the supernatant by pipette or by aspiration.

Note：**In the process of removing the supernatant, do not take away the magnetic beads.**

Note：**Be careful not to have any liquid residue on the cap and wall of the tube as well as the bottom of the tube.**

6.4. Elute the DNA/RNA

1. Add 100 μL of Elution Buffer to each tube, then close the cap.

2. Vortex the tubes for 20 seconds at high speed, centrifuge the tubes briefly, then incubate the tubes at 80℃ for 5 minutes.

3. Place the tubes in the magnetic stand, then let the tubes stand for 1 minute.

4. Without disturbing the Magnetic Beads, transfer the liquid phase containing the eluted DNA/RNA to a new nonstick 1.5-mL microcentrifuge tube.

5. Store eluted DNA/RNA for up to 6 hrs on ice, or up to 24 hrs at −20°C.

7. Trouble Shooting

Observation	Possible cause	Action
Poor extraction efficiency (low yields)	1. Magnetic stand does not match.	Magnetic stand(Cat. No. M24040801) from Ducky Bio are recommended for better recovery rates.
	2.The volume of positive control standard dilution to the test sample is less than the amount of DNA measured in the test sample without the addition of the DNA/RNA control.	Add a volume of positive control standard dilution to each test sample so that the total DNA/RNA amount is 2-10 times the amount of DNA/RNA measured in the test sample without the addition of the DNA/RNA control.
	3、When removing the supernatant in the Bind the DNA step and Wash the DNA step, the supernatant is not completely removed, especially the cap and wall of the tube.	In the process of removing the supernatant, do not take away the magnetic beads. Be careful not to have any liquid residue on the cap and wall of the tube as well as the bottom of the tube.
	4、The amount of pipette in the bead or sample is not accurate.	Votex them throughly, the appearance of the mixture should be homogeneous, especially the bottom sediment.
	5、The Magnetic beads were frozen, or they were removed when the supernatant was removed.	If the magnetic beads have been frozen, it is recommended to contact Ducky for a new magnetic bead. Be careful not to remove the magnetic beads while you're removing the supernatant.
	6、Special sample type.	we suggest that you send the sample to us, and we would like to customize a new Sample Preparation Kit for your sample.

8. Safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on).

8.1. Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Resources" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container.
- Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.

➢ Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.

➢ Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

➢ IMPORTANT! Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

WARNING! 4L Reagent and Waste Bottle Safety. Four-liter reagent and waste bottles can crack and leak. Each 4-liter bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position.

8.2. Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases.

Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

· U.S. Department of Health and Human Services, Biosafety in Microbiological and Biomedical Laboratories (BMBL), 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
https://www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P. pdf

· Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
www.who.int/publications/i/item/9789240011311

9. Customer and technical support

Visit Duckybio.com/support for the latest service and support information.

- Worldwide contact telephone numbers：+86 18039226538

– Email：sales@duckybio.com

– Website：www.duckybio.com

- Product support information

– Product FAQs

– Software, patches, and updates

– Training for many applications and instruments

- Order and web support

- Product documentation

– User guides, manuals, and protocols

– Certificates of Analysis

– Safety Data Sheets (SDSs; also known as MSDSs)