resDetect™ resDNA Sample Preparation Kit II (Magnetic Beads)

Catalog Number：OPA-R024

Assay Tests: 50 Preps

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures.
IMPORTANT: Please carefully read this user guide before performing your experiment.

Contents List

Product Information

The resDNA Sample Preparation Kit II is designed for extraction of residual DNA (resDNA) from biopharmaceuticals. The resDNA Sample Preparation Kit II is suitable for use in combination with the resDetect™ HEK293/HEK293T/*Pichia pastoris* resDNA Quantitation Kit (qPCR). It is not compatible with other resDNA Quantitation kits. Before detecting the residual DNA content, use the resDNA Sample Preparation Kit to extract nucleic acids from test samples. For detection information, see the resDNA Quantitation Kit User Guide (ACROBiosyestems.com).

This kit is isolate DNA from a sample using magnetic beads. The process typically involves lysing the sample to release the DNA, then using magnetic beads coated with a DNA-binding agent to selectively bind the DNA. The beads are then separated from the mixture using a magnetic stand, and the DNA can be washed and eluted off the beads for further analysis or use. This method is often preferred over traditional methods due to its high efficiency and ease of use.

This instruction included the process of manual DNA extraction and automated DNA extraction. About automatic DNA extraction, we provide a program for KingFisher™ Flex.

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Contents and Storage

The kit can be used for 50 preps of DNA extraction from test samples.

Contents	Amount	Storage
Buffer NT	1.5 mL	10℃ to 30℃ **Note**: The Proteinase K and MagBeads Suspension can be stored in ambient temperature (10 to 30℃). For optimal long-term stability, these two components are recommended to be stored at 2℃ to 8℃.
Buffer LA	1.5 mL	
Buffer LB	24 mL	
Proteinase K	4 mL	
MagBeads Suspension (MB)	1.5 mL	
CR Powder	310 μg	
Buffer WA	38 mL	
Buffer WB	18 mL	
Buffer EB	6 mL	
Sample Dilution Buffer	10 mL	

The unopened reagents can be stored stably for 12 months from the date of manufacture when kept at a storage temperature between 10℃ and 30℃.

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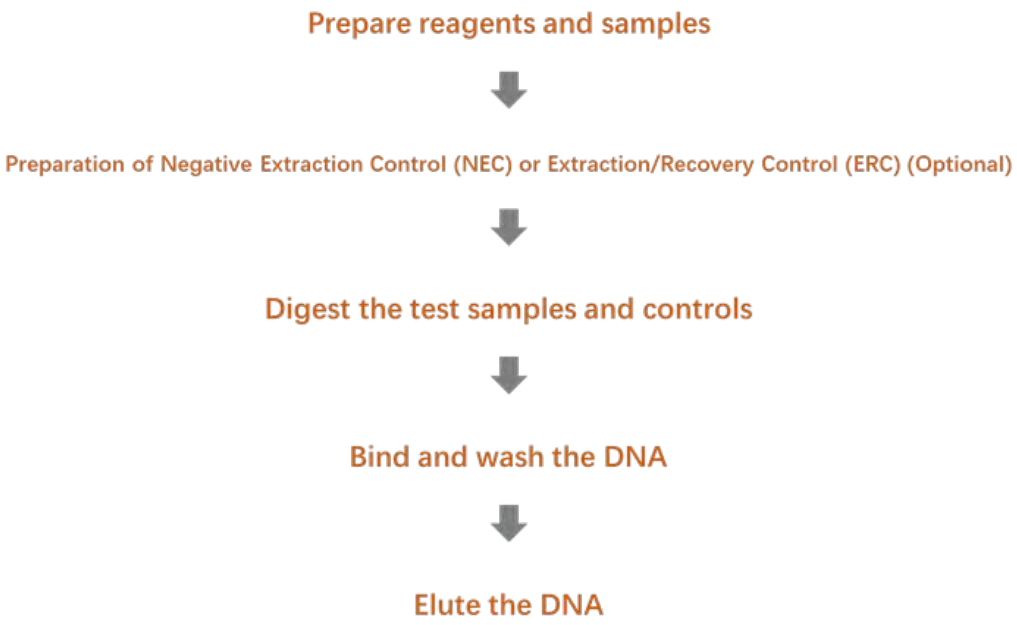
PART A: Manual DNA extraction

Required materials not supplied

Equipment	Magnetic stand
	Block heater
	Mini centrifuge
	Vortex
	Pipettors: P1000, P200, P100, P10
Reagents	Isopropanol, 99.7%
	Ethanol, 99.7%
	1×PBS (free of Mg^{2+} and Ca^{2+}) or 1×TE (pH7.0-pH8.0) as sample dilution buffer
	DNase/RNase-free ddH_2O
Consumables	Disposable gloves
	Nuclease-free, DNA-free aerosol-resistant pipet tips
	Low DNA-Binding Microcentrifuge Tubes (Nuclease-free, DNA-free)

Workflow for manual DNA extraction



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Prepare the reagents and samples for manual DNA extraction

1. Prepare the reagents: before first use of the kit.

1.1 Incubate the MagBeads Suspension at room temperature for 30 min in advance. Before being used, the magnetic beads have to be vigorous vortexed and mixed.

1.2 Refer to the bottle label, add 40mL of 99.7% ethanol to the bottle of Buffer WA, then mix completely.

1.3 Refer to the bottle label, add 80mL of 99.7% ethanol to bottle of Buffer WB, then mix completely.

1.4 Label the bottle to indicate that it contains ethanol, then store the bottle at room temperature.

1.5 Preparation of CR Solution: Briefly centrifuge the CR Powder tube, then add 310 µL DNase/RNase-free ddH_2O to the tube, and vortex thoroughly.

NOTE: (1) **Don't add CR Solution when extracting Pichia pastoris resDNA.**

(2) The CR Solution should be stored at - 20℃, and aliquot the CR solution into small volumes to avoid freeze - thaw cycles.

2. Prepare the samples.

2.1 Sample dilution (if necessary)

If the test samples are from the upstream or midstream products of the biopharmaceutical process, they may contain relatively high levels of DNA residues. To ensure the accuracy of the test and keep the sample's measured value within the linear range of the standard curve, the sample diluent should be used to perform serial dilutions of the samples by a factor of 10 to 1000 before proceeding with the next step. Dilute test samples with sample dilution buffer before DNA extraction. 1×PBS (free of Mg^{2+} and Ca^{2+}) or 1×TE (pH7.0-pH8.0) can also be used as sample dilution buffer.

2.1.1 If the samples have been diluted, the sample diluent should be used as a negative control.

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2.1.2 For the powder testing samples, please dissolve the samples with sample dilution buffer.

3. Prepare the NEC and ERC. (Optional)

3.1 Preparation of Negative Extraction Control (NEC)

A Negative Extraction Control (NEC) omits any DNA template from a reaction. This control is used to monitor contamination during nucleic acid extraction. In cases where large numbers of DNA samples need extracted, it is recommended that negative extraction controls are included between the samples for testing.

3.1.1 Label low DNA-binding 1.5 mL microfuge tubes as "NEC".

3.1.2 Add **100 µL** of 1×PBS (free of Mg^{2+} and Ca^{2+}) or 1×TE (pH7.0~pH8.0) to each tube.

NOTE: NEC should be the same as sample dilution buffer (If used in the process sample dilution).

3.2 Preparation of Extraction/Recovery Control (ERC) (Optional)

An Extraction/Recovery Control (ERC) can be utilized to evaluate the efficiency of DNA extraction, recovery, and quantitation from test samples. Moreover, the ERC can be used to verify assay and system performance.

NOTE: Adjust the amount of target residual DNA control added to the sample for those test samples that exhibit higher background DNA levels. To ensure accurate results, the amount of target residual DNA control added to a test sample should be approximately two to three times the quantity of DNA measured in the test sample prior to the addition of the target residual DNA control. To calculate the efficiency of DNA recovery and quantification from the test samples, subtract the amount of DNA measured in the sample before adding the target residual DNA control from the amount of DNA measured in the ERC sample.

To learn about the procedure preparing ERC sample containing target residual DNA

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control per well for qPCR analysis, refer to the corresponding User Guide of resDNA Quantitation Kit (ACROBiosyestems.com).

Protocol for manual DNA extraction

1. Digest the test samples and controls.

1.1 Label low DNA-binding 1.5/2.0mL microfuge tubes as “Sample”,and “NEC”.

1.2 Add 100 µL of samples and controls to each tube.

1.3 Add 22 µL of Buffer NT, 70 µL of Proteinase K and 25 µL of Buffer LA to each tube, briefly vortex and centrifuge.

1.4 Incubate at 56°C for 30 min on a block heater, with vortexing at 1000 rpm. If available, set heater lid at 70°C.

1.5 Briefly centrifuge, and cool samples to room temperature.

1.6 Add 400 µL of Buffer LB to each tube, then close the cap and invert five times to mix.

1.7 Vortex 1 min and briefly centrifuge.

2. Bind the DNA

2.1 Add 180 µL of isopropanol, 25 µL of MagBeads Suspension and 3 µL of CR Solution to each tube, then close the cap and invert five times to mix.

NOTE: The MagBeads Suspension should be resuspended before used. If extract the *Pichia pastoris* DNA, do not add CR solution.

2.2 Vortex all the tubes for 1 min.

2.3 Let the tubes stand for 5 min, and then vortex for 30 seconds.

2.4 Repeat the step 2.3.

2.5 Briefly centrifuge and place the tubes in the magnetic stand with the pellet against the magnet, then let the tubes stand for 5 min or until the solution is clear.

2.6 Without disturbing the magnetic beads, remove the supernatant using a pipette or by aspiration.

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3. Wash the DNA

3.1 Add 700 µL Buffer WA to each tube, then vortex for 10 seconds.

3.2 Briefly centrifuge the tubes, then place the tubes in the magnetic stand, let the tubes stand for 2 min or until the solution is clear.

3.3 Without disturbing the magnetic beads, remove the supernatant using a pipette or by aspiration.

3.4 Repeat the steps 3.1-3.3.

3.5 Add 700 µL Buffer WB to each tube, then vortex for 10 seconds.

3.6 Repeat the steps 3.2-3.3.

3.7 Use a P10 pipettor to remove the remaining solution from the bottom of the tube.

3.8 With the tube lid open, air-dry the Magnetic beads in the magnetic stand for no more than 5 minutes at room temperature.

NOTE: Do not over-dry, or the bonded DNA are not easily eluted.

4. Elute the DNA

4.1 Add 50-100 µL of Buffer EB to each tube, then resuspend the beads by vortexing or pipetting up and down until suspension is fully homogenized.

4.2 Incubate the tubes at 70°C for 10 min on a block heater, with vortexing at 1000 rpm.

4.3 Briefly centrifuge the tubes for 15 seconds, then place the tubes in the magnetic stand, let the tubes stand for 2-5 min or until the solution is clear.

4.4 Use a P100 pipettor to transfer the liquid phase to a new 1.5 mL microcentrifuge tube.

NOTE: Do not disturb the magnetic beads.

5. DNA Sample Storage

5.1 The purified, high-quality eluted DNA is ready to use in demanding downstream applications. Store eluted DNA for up to 24 hours at 2°C to 8°C or for long time at −20°C.

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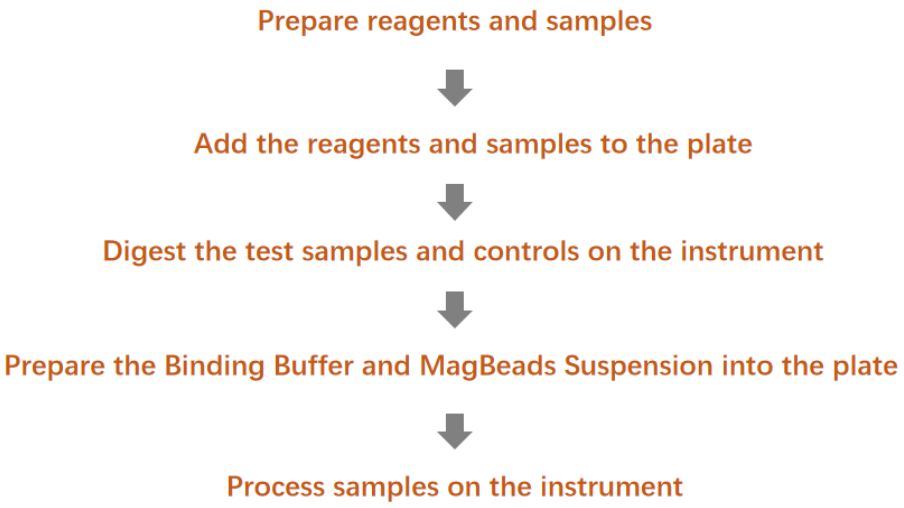
PART B: Automated DNA extraction: Apply to KingFisher™ Flex

Required materials not supplied for KingFisher™ Flex

Equipment	Mini centrifuge
	Vortex
	Automated extraction instrument (KingFisher™ Flex)
	Pipettors: P1000, P200, P100, P10
Reagents	Isopropanol, 99.7%
	Ethanol, 99.7%
	1× PBS (free of Mg^{2+} and Ca^{2+}) or 1×TE (pH7.0-pH8.0) as sample dilution buffer
	DNase/RNase-free ddH_2O
Consumables	Disposable gloves
	Nuclease-free, DNA-free aerosol-resistant pipet tips
	Low DNA-Binding Microcentrifuge Tubes (Nuclease-free, DNA-free), 96 Deep-well plate, 96-Strip Tip Comb

Workflow for automated DNA extraction by KingFisher™ Flex



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Prepare the reagents and samples for DNA extraction by KingFisher™ Flex

1. Prepare the reagents: before first use of the kit.

1.1 Please refer to manual DNA extraction, point 1 on page 4.

2. Prepare the samples.

2.1 Please refer to manual DNA extraction, point 2 on page 4.

3. Prepare the NEC and ERC. (Optional)

3.1 Please refer to manual DNA extraction, point 3 on page 5.

Protocol for DNA extraction by KingFisher™ Flex

The following steps apply for KingFisher™ Flex (96-well plate).

1. Reagents dispensing

1.1 Prepare 6 KingFisher™ Flex 96 deep-well plates, label the plates as: **96 tip comb plate**, **Lysis plate**, **Wash1 plate**, **Wash2 plate**, **Wash3 plate** and **Elute plate**.

1.2 Put a KingFisher™ Flex 96-strip tip comb into the 96 tip comb plate.

1.3 Add 100 µL of samples, 22 µL of Buffer NT, 70 µL of Proteinase K and 25 µL of Buffer LA to each well in the Lysis plate.

NOTE: (1) Transfer the whole volume of ERC sample into Lysis plate for extraction. Preparation of ERC samples could refer to the corresponding user guide of resDNA Quantitation Kit (ACROBiosystems.com).

(2) Proteinase K, Buffer NT and Buffer LA cannot be premixed together, they should be added separately.

1.4 Add 700 µL of Buffer WA to each well in Wash1 plate.

1.5 Add 700 µL of Buffer WA to each well in Wash2 plate.

1.6 Add 700 µL of Buffer WB to each well in Wash3 plate.

1.7 Add 100 µL of Buffer EB to each well in Elute plate.

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2. Automated Extraction Process

2.1 Clean the work space with 75% ethanol before use.

2.2 Open the software **Thermo Scientific BindIt**, and open the procedure **Acro-OPA-R024.bdz**. Click "**Start**".

2.3 Put the plate in the specified sequence: **96 tip comb plate, Elute plate, Wash3 plate, Wash2 plate, Wash1 plate, Lysis plate**.

NOTE: Please check all the plates are placed correctly and the 96-strip tip comb are inserted before running.

2.4 Run the procedure.

2.5 Prepare the binding buffer according to the number of samples to be tested. Prepare a 50 mL tube for binding buffer using reagents and volumes shown in the table below.

IMPORTANT! To compensate for pipetting losses, it is recommended that the **N** is equals to the number of extracted samples plus 1 or 2.

Kit Reagents	Volume for 1 sample	Volume for Binding Buffer
Buffer LB	400 µL	400 µL×**N**
Isopropanol	180 µL	180 µL×**N**
CR Solution	3 µL	3 µL×**N**
Total	583 µL	583 µL×**N**

2.6 After the sample digestion is finished, take out the Lysis plate, and add 583 µL of binding buffer, 25 µL of MagBeads Suspension to each well in Lysis plate.

NOTE: Mix the binding buffer and MagBeads Suspension fully (at least 1 minute) before dispensing. Resuspend the MagBeads suspension by dispensing every 5~8 wells.

2.7 Put the Lysis plate back and click "Start" and run the procedure.

2.8 After the procedure is finished, take out the Elute plate and transfer the eluted DNA

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to new 1.5 mL low DNA-binding microcentrifuge tubes or PCR tubes immediately. The purified, high-quality eluted DNA is ready to use in demanding downstream applications.

NOTE: Store eluted DNA for up to 24 hours at 2℃ to 8℃ or for long time at −20℃.

2.9 Take out other plates and clean the work space with 75% ethanol.

NOTE: The interval between extraction experiments is recommended above 30 minutes to avoid cross contamination.

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