resDetect™ resDNA Sample Preparation Kit (Magnetic Beads)

Catalog Number：OPA-R005

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

Product Information

The resDNA Sample Preparation Kit is designed for extraction of residual DNA (resDNA) from biopharmaceuticals. The resDNA Sample Preparation Kit is suitable for use in combination with the resDetect™ *E. coli*/CHO/E1A/E1A&SV40LT/Plasmid 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 (ACROBiosystems.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	
1×PBS	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℃.

In an environment with a low temperature (< 20℃), precipitates may form in Buffer LA, Buffer LB and Buffer WA. Place the reagent bottles in a water bath at 37℃-50℃ for about 10 minutes until the precipitates are completely dissolved before use.

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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)

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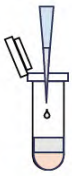
Workflow for manual DNA extraction

Step 1 Digest samples



+100 µL Sample
+22 µL Buffer NT
+70 µL Proteinase K
+25 µL Buffer LA
for 10 s

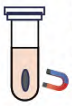
56°C, 1000rpm, 30 min
6000 rpm for 10 s

Step 2 Bind the DNA

+400 µL Buffer LB
for 1 min
6000 rpm for 10 s

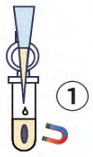
+180 µL Isopropanol
+25 µL Magbeads Suspension
+3 µL CR Solution
for 1 min

Let the tubes stand for 10 min, vortex for 30 s in every 5 min.
6000rpm for 10 s

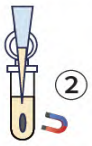


Put tubes in the magnetic stand for 5 min.
Remove the supernatant.

Step 3 Wash the DNA



+700 µL Buffer WA
for 10 s
6000 rpm for 10 s



Put tubes in the magnetic stand for 2 min.
Remove the supernatant.

Repeat steps ① and ② of Buffer WA washing procedure.

+700 µL Buffer WB
for 10 s
6000 rpm for 10 s

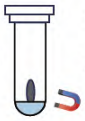
Put tubes in the magnetic stand for 2 min.
Remove the supernatant.

Remove solution and air-dry the magnetic beads for 2-5min.

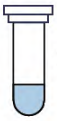
Step 4 Elute the DNA

+100 µL Buffer EB
for 15~20 s

70°C, 1000rpm, 10 min
6000 rpm for 15 s



Let the tubes stand for 2-5 min.
Transfer DNA to a new tube.



DNA ready for qPCR

+ add vortex centrifuge incubate

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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 minutes in advance. Before being used, the magnetic beads have to be vigorous vortexed and mixed.

NOTE: The MagBeads Suspension should be mixed for at least 1 minute until they are evenly dispersed.

Invert the tube several times and observe to confirm the state of the magnetic beads: There should be no magnetic beads adhering to the bottom of the tube, and the magnetic bead suspension should be in a uniformly dispersed state with a consistent color and no dark-colored aggregated lumps.

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

1.3 Refer to the bottle label, add 80 mL 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: The CR Solution should be stored at -20℃, and aliquot the CR solution into small volumes to avoid more than three 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

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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.

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 to be 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 of 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

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

NOTE: Briefly centrifuge: It is recommended to centrifuge at 3000-6000 rpm for 5-10 seconds. It is applicable to all of briefly centrifuge mentioned in this instruction manual.

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: (1) To ensure the accuracy of the magnetic beads volume added, please vortex the MagBeads Suspension for at least 1 minute before use.

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(2) After adding 3-4 samples, please vortex the MagBeads Suspension again for about 30 seconds before continuing to add samples.

(3) To ensure uniformity, it is recommended that when aspirating the magnetic beads each time, you can gently pipette up and down twice with a pipette before aspiration.

2.2 Vortex all the tubes for 1 min.

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

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.

3. Wash the DNA

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

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 s.

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: (1) Residual ethanol will interfere with the subsequent PCR reaction, please ensure that the ethanol is completely volatilized. Do not over-dry, or the

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bonded DNA are not easily eluted.

(2) Observe the magnetic beads under bright light, add the Buffer EB immediately when the surface of the magnetic beads appears dull.

(3) If the surface of the magnetic beads is yellowish-brown, it indicates excessive drying, which will affect the recovery rate of nucleic acid.

4. Elute the DNA

4.1 Add **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 s, 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 be used in demanding downstream applications. It is recommended to finish qPCR testing within 24 hours, and the DNA can be temporarily stored at 2°C to 8°C. For long-term storage, it should be placed at −20°C, and you should complete the DNA testing within 7 days.

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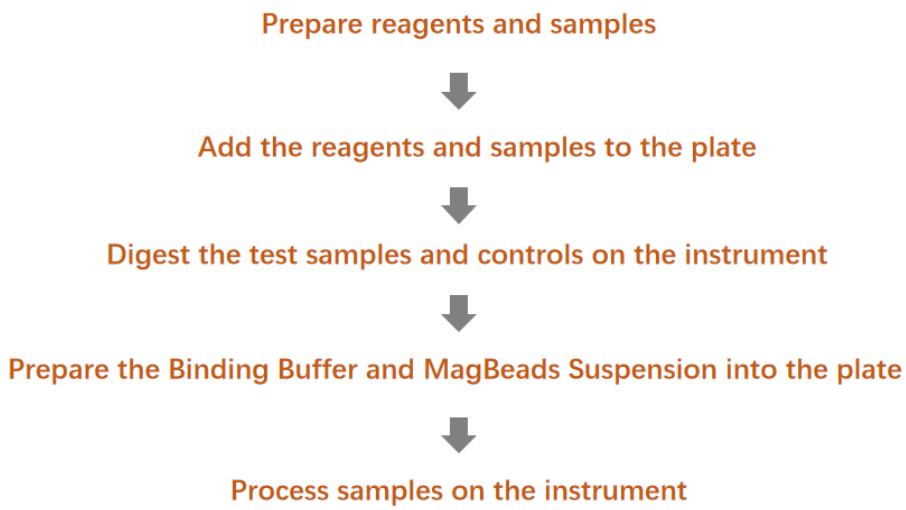
PART B: Automated DNA extraction: Apply to KingFisher™ Flex

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 5.

2. Prepare the samples.

2.1 Please refer to manual DNA extraction, point 2 on page 5.

3. Prepare the NEC and ERC. (Optional)

3.1 Please refer to manual DNA extraction, point 3 on page 6.

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: It is recommended to finish qPCR testing within 24 hours, and the DNA can be temporarily stored at 2℃ to 8℃. For long-term storage, it should be placed at −20℃, and you should complete the DNA testing within 7 days. A multi-channel pipette helps to transfer liquid quickly.

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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Appendixes

Appendix A: FAQs

Common Questions	Possible reasons & Actions
Do precipitates appearing in Buffer LA, Buffer LB and Buffer WA affect extraction efficiency?	Precipitates appearing in Buffer LA, Buffer LB and Buffer WA may be caused by low temperature. Please dissolve the precipitates according to the instruction of part "Contents and Storage" on page 2. It has no effect on the extraction efficiency after the precipitates are completely dissolved. The laboratory temperature should be kept at 20℃ to 30℃ day and night, and the temperature should not be lower than 20℃ to avoid precipitates appearing in Buffer LA, Buffer LB and Buffer WA.
How long can the eluted DNA be stored without affecting the testing results?	It is recommended to finish qPCR testing on the same day as extraction or within 24 hours. The DNA can be temporarily stored at 2°C to 8°C. For long-term storage, it should be placed at −20°C, and you should complete the DNA testing within 7 days. Also, it should be frozen and thawed only once.
Does the kit stored at room temperature affect the extraction efficiency?	The kit has been verified and can be stored at room temperature (10-30°C) for one year. For optimal long-term stability, the MagBeads Suspension and Proteinase K are recommended to be stored at 2℃ to 8℃.
What could be done if the extraction efficiency of high-protein samples is poor?	For samples with high protein concentration, you can try to extend the digestion time of the samples with Proteinase K from 30 minutes to 1 hour.

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What is the typical elution volume?	It is recommended that the elution volume be 100 µL, which helps maximize the efficiency of extraction.

Appendix B: Consumables Recommendation

Consumables	Catalog Number	Manufacturer
2.0 mL Low DNA-Binding Microcentrifuge Tubes	72.695.700	SARSTEDT
1.5 mL Low DNA-Binding Microcentrifuge Tubes	72.706.700	SARSTEDT

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