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.

Product Information

The resDNA Sample Preparation Kit is designed for extraction of residual DNA (resDNA) from biopharmaceutical productions that are produced in cell lines such as *E. coli*, CHO, E1A, SV40LT, and residual plasmid DNA. Use the kit before you detect resDNA from test samples. For detection information, see the resDNA Quantitation Kit User Guide (ACROBiosyestems.com).

This manual covers manual DNA extraction instructions and automated DNA extraction instructions (Applicable to KingFisher™ Flex).

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.

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 in 2-8℃.
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	

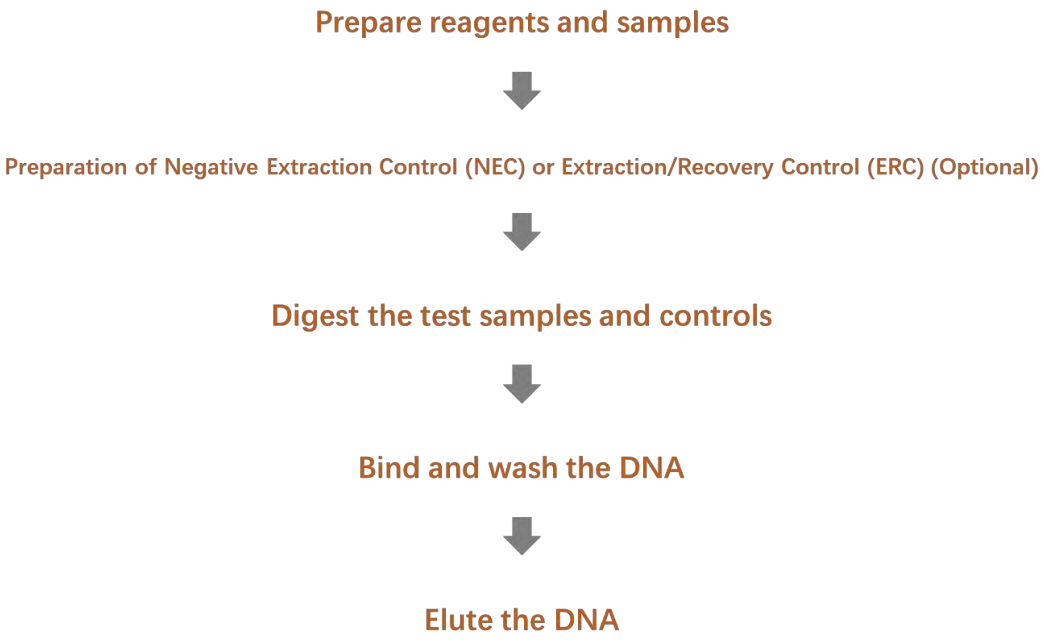
The unopened kit is stable for 18 months from the date of manufacture if stored at 10°C to 30°C.

Manual DNA extraction

Required materials not supplied.

Equipments	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



Prepare the reagents and samples.

Prepare the reagents: before first use of the kit.

1. Incubate the MagBeads Suspension at room temperature for 30 mins, or until the beads are completely suspended.
2. Refer to the bottle label, add amount of 99.7% ethanol to bottle of Buffer WA or WB, then mix completely.
3. Label the bottle to indicate that it contains ethanol, then store the bottle at room temperature.
4. 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℃, it can be divided into small portions to avoid freeze-thaw cycles.

Prepare the samples. Sample dilution (if necessary)

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

1. Dilute test samples before DNA extraction with sample dilution buffer. Please use 1×PBS (free of Mg^{2+} and Ca^{2+}) or 1×TE (pH7.0~pH8.0) as sample dilution buffer.
2. For the powder testing samples, please resolve the samples with sample dilution buffer.

Prepare the NEC and ERC. (Optional)

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.

1. Label low DNA-binding 1.5 mL microfuge tubes “NEC”.
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).

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

You can use an Extraction/Recovery Control (ERC) to assess the efficiency of DNA extraction, recovery, and quantitation from test samples. Additionally, you can use ERC to verify assay and system performance.

NOTE: Adjust the amount of Target residual DNA control added to the sample for those test samples that contain higher background levels of DNA. To ensure accurate results, the amount of Target residual DNA control that you add to a test sample should be approximately two to three times the amount of DNA measured in the test sample without the addition of the Target residual DNA control. To calculate the efficiency of DNA recovery and quantitation from the test samples, subtract the amount of DNA measured in the sample without the addition of 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 (ACROBiosyestems.com).

Digest the test samples and controls.

1. Label low DNA-binding 1.5/2.0mL microfuge tubes “Sample”, “NEC”.
2. Add 100 µL of samples and controls to each tube.
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.
4. Incubate at 56°C for 30 mins on a block heater, with vortexing at 1000 rpm. If available, set heater lid to 70°C.
5. Briefly centrifuge, and cool samples to room temperature.
6. Add 400 µL of Buffer LB to each tube, then close the cap and invert five times to mix.
7. Vortex 1 min and briefly centrifuge.

Bind the DNA

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 use.
2. Vortex all the tubes for 1 min.
3. Let the tubes stand for 5 mins, and then vortex for 30 seconds.
4. Repeat the step 3.
5. Briefly centrifuge and place the tubes in the magnetic stand with the pellet against the magnet, then let the tubes stand for 5 mins or until the solution is clear.
6. Without disturbing the magnetic beads, remove the supernatant using a pipette or by aspiration.

Wash the DNA

1. Add 700 µL Buffer WA to each tube, then vortex for 10 seconds.
2. Briefly centrifuge the tubes, then place the tubes in the magnetic stand, let the tubes stand for 2 mins or until the solution is clear.
3. Without disturbing the magnetic beads, remove the supernatant using a pipette or by aspiration.
4. Repeat the steps 1-3.
5. Add 700 µL Buffer WB to each tube, then vortex for 10 seconds.
6. Repeat the steps 2-3.
7. Use a P10 to remove the remaining solution from the bottom of the tube.

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; the bonded DNA are not easily eluted from the over-dried beads.

Elute the DNA

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.
2. Incubate the tubes at 70°C for 10 mins on a block heater, with vortexing at 1000 rpm.
3. Briefly centrifuge the tubes for 15 seconds, then place the tubes in the magnetic stand, let the tubes stand for 2-5 mins or until the solution is clear.
4. Use a P100 to transfer the liquid phase to a new 1.5 mL microcentrifuge tube.

   NOTE: Do not disturb the magnetic beads.

   The purified, high-quality eluted DNA is ready to use in demanding downstream applications.

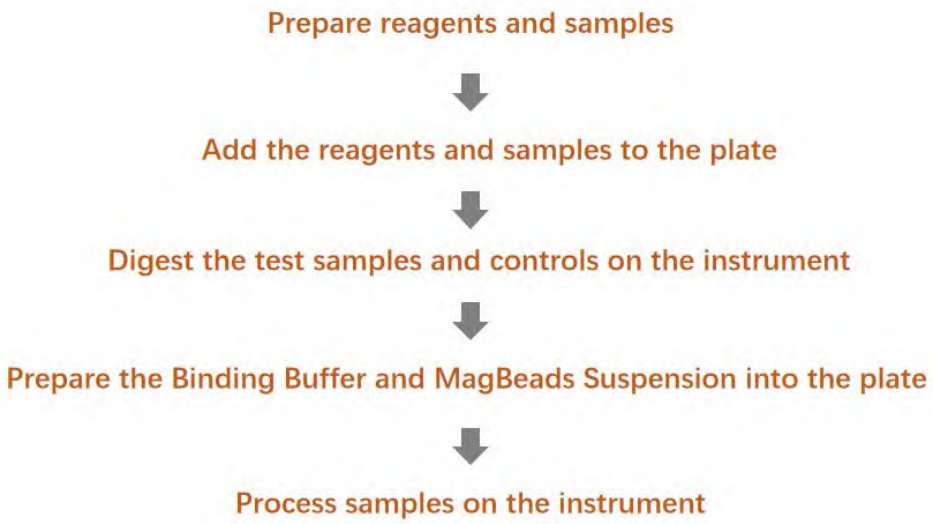
   Store eluted DNA for up to 24 hours at 2°C to 8°C or for long time at −20°C.

Automated DNA extraction:Apply for KingFisher™ Flex

Required materials not supplied.

Equipments	Mini centrifuge
	Vortex
	Automated extraction instrument (KingFisher™ Flex)
	Pipettors: P1000, P200, P100, P10
Reagents	Isopropanol, 99.7%
	Ethanol, 99.7%
	1X 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



Prepare the reagents and samples.

Prepare the reagents: before first use of the kit.

1. Incubate the MagBeads Suspension at room temperature for 30 mins, or until the beads are completely suspended.
2. Refer to the bottle label, add amount of 99.7% ethanol to bottle of Buffer WA or WB, then mix completely.
3. Label the bottle to indicate that it contains ethanol, then store the bottle at room temperature.
4. 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℃, it can be divided into small portions to avoid freeze-thaw cycles.

Prepare the samples.

Sample dilution (if necessary)

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

1. Dilute test samples before DNA extraction with sample dilution buffer. Please use 1X PBS (free of Mg^{2+} and Ca^{2+}) or 1 X TE (pH7.0~pH8.0) as sample dilution buffer.
2. For the powder testing samples, please dissolve the samples with sample dilution buffer.

Reagents dispensing

1. Prepare 6 KingFisher 96 deep-well plates, label the plates as: **96 tip comb plate**, **Lysis plate**, **Wash1 plate**, **Wash2 plate**, **Wash3 plate** and **Elute plate**.
2. Put a KingFisher 96-strip tip comb into the **96 tip comb plate**.
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: Tranfer the whole volume of ERC sample into Lysis plate for extraction. Preparation of ERC samples could be refer to the corresponding user guide of resDNA Quantitation Kit (ACROBiosystems.com).

   Proteinase K, Buffer NT and Buffer LA cannot be premixed together, they should be added separately.
4. Add **700 µL of Buffer WA** to each well in **Wash1 plate**.
5. Add **700 µL of Buffer WA** to each well in **Wash2 plate**.
6. Add **700 µL of Buffer WB** to each well in **Wash3 plate**.
7. Add **100 µL of Buffer EB** to each well in **Elute plate**.

Automated Extraction Process

The following steps applies for KingFisher™ Flex (24074420).

1. Clean the work space with 75% ethanol before use.
2. Open the software **KingFisher BinIt**, and open the procedure **Acro-OPA-R005.bdz**. Click "**Start**".
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.
4. Run the procedure.
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 of 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**

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.

7. Put the **Lysis plate** back and click "**Start**" and run the procedure.
8. After the procedure is finished, take out the **Elute plate** and transfer the eluted DNA 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°C to 8°C or for long time at −20°C.

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.