



resDetect™ HEK293 /HEK293T resDNA Sample Preparation Kit (Magnetic Beads)

User Guide: V1.0

Catalog Number: OPA-R033

Assay Tests: 25 Preps

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

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Product Information

The HEK293 /HEK293T resDNA Sample Preparation Kit is designed for the extraction of residual DNA (resDNA) from biopharmaceuticals. The resDNA Sample Preparation Kit is suitable for use in combination with the resDetect™ HEK293 /HEK293T resDNA Ultra-Low Quantitation Kit (qPCR). It is not compatible with other resDNA Quantitation kits. Before detecting the residual DNA content, the HEK293 /HEK293T resDNA Sample Preparation Kit should be used to extract nucleic acids from test samples. For more information about the detection kits, please refer to the resDNA Quantitation Kit User Guide (ACROBiosystems.com).

This kit is used to 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 HEK293 /HEK293T resDNA Sample Preparation Kit can be compatible with the Automated Nucleic Acid Extraction System (ACRO Cat. No.: OPE-32S) and KingFisher™ Flex. Please visit the web ACROBiosystems.com to get more information about the Automated Nucleic Acid Extraction System.

Contents and Storage

The kit can be used for 25 preps of DNA extraction from test samples.

Contents	Amount	Storage
Buffer LA	650 µL	10°C to 30°C Note: The Proteinase K and MagBeads Suspension can be stored in ambient temperature (10 to 30°C). For optimal long-term stability, these two components are recommended to be stored at 2°C to 8°C.
Buffer LB	10 mL	
Proteinase K	2 mL	
MagBeads Suspension	650 µL	
CR Powder	310 µg	
Buffer WA	18 mL	
Buffer WB	8 mL	
Buffer EB	3 mL	
Sample Dilution Buffer	6 mL	

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

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

List of Abbreviations

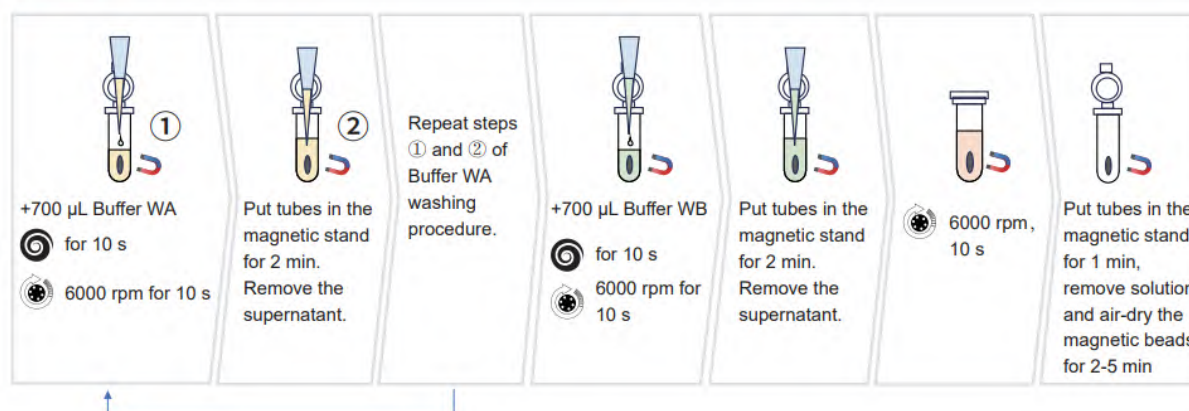
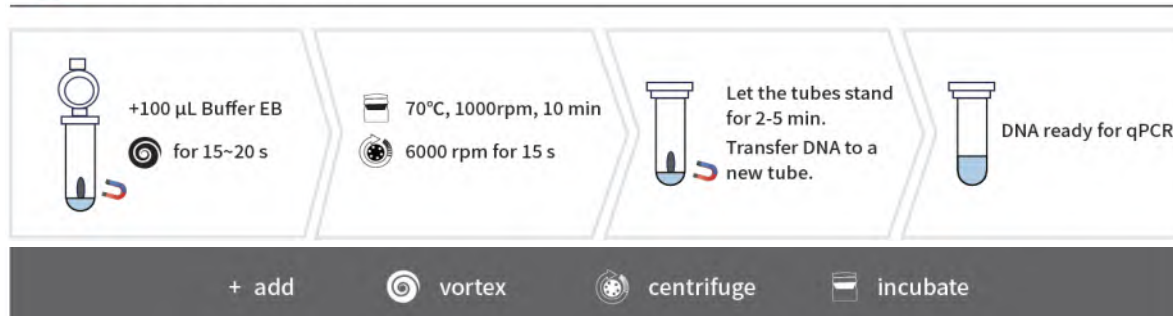
Abbreviations	Full Name
HEK293	Human Embryonic Kidney 293
ERC	Extraction/Recovery Control
NEC	Negative Extraction Control
SERC	Sample Extraction/Recovery Control

PART A: Manual DNA extraction

Required materials not supplied

Equipment	Magnetic stand
	Block heater
	Mini centrifuge
	Vortex
	Pipettors: P1000, P200, P100, P10
Reagents	Ethanol, 99.7%
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

Step 1 Digest samples**Step 2** Bind the DNA**Step 3** Wash the DNA**Step 4** Elute the DNA

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 20 mL of 99.7% ethanol to the bottle of Buffer WA, then mix completely.
- 1.3 Refer to the bottle label, add 40 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 Water (it is provided in the kit OPA-R031 or OPA-R032) to the tube, and vortex thoroughly.

NOTE: The CR Solution should be stored at -20°C, and aliquot the CR solution into small volumes to avoid more than three freeze-thaw cycles.

2. Prepare the samples.

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 performing DNA extraction.

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

2.2 Preparation of the test sample: Label low DNA-binding 1.5/2.0 mL microfuge tubes as "**Sample**", and add **100 µL** of the test sample to each tube.

3. Prepare the NEC, ERC and SERC. (Optional)

3.1 Preparation of NEC

A 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 1.5/2.0 mL microfuge tubes as "**NEC**".

3.1.2 Add **100 µL** of Sample Dilution Buffer 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 ERC and SERC (Optional)

An ERC and SERC 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. Taking a spiking amount of 6 pg DNA as an example:

3.2.1 Preparation of ERC: Label low DNA-binding 1.5/2.0 mL microfuge tube as "**ERC**", and add 100 µL of Sample Dilution Buffer to each tube. Then add 20 µL of DNA (300 fg/µL) to each ERC tube, vortex gently.

3.2.2 Preparation of SERC: Label a low DNA-binding 1.5/2.0 mL microfuge tube as "**SERC**", and add 100 µL of the test sample to each tube. Then add 20 µL of DNA (300 fg/µL) to each SERC tube, vortex gently.

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 SERC sample.

Protocol for manual DNA extraction

1. Digest the test samples and controls.

- 1.1 Label low DNA-binding 1.5/2.0 mL microfuge tubes as “Sample”.
- 1.2 Prepare the samples and controls according to “**2. Prepare the Samples**” on page 5 and “**3. Prepare the NEC, ERC and SERC**” on page 6.
- 1.3 Add **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.

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 **400 µL of Buffer LB** to each tube, then close the cap and invert five times to mix.
- 2.2 Vortex the tubes for 1 min and briefly centrifuge.
- 2.3 Add **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.

(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.

- 2.4 Vortex all the tubes for 1 min.
- 2.5 Let the tubes stand for 5 min, and then vortex for 30 s.
- 2.6 Repeat the step 2.5.
- 2.7 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.
- 2.8 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 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.

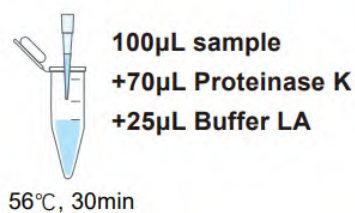
PART B: Automated DNA extraction: Apply to ACRO OPE-32S

Required materials not supplied for ACRO OPE-32S

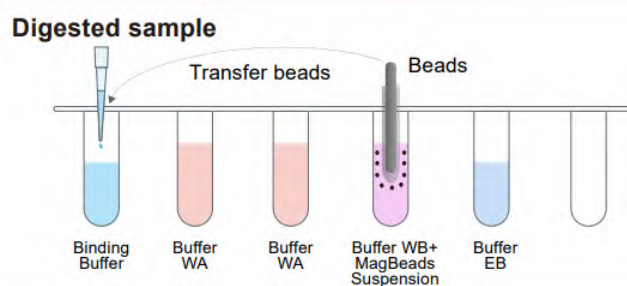
Equipment	Automated Nucleic Acid Extraction System (OPE-32S)
	Mini centrifuge
	Vortex
	Block heater
	Pipettors: P1000, P200, P100, P10
Reagents	Ethanol, 99.7%
Consumables	Disposable gloves
	Nuclease-free, DNA-free aerosol-resistant pipet tips
	Low DNA-Binding Microcentrifuge Tubes (Nuclease-free, DNA-free)
	96 V-Bottom Deep-well plate (Cat. No.: OPA-R013)
	8-Strip V-Bottom Tip Comb (Cat. No.: OPA-R014)

Workflow for automated DNA extraction by ACRO OPE-32S

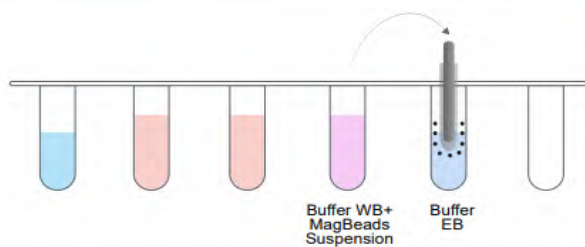
1 Digest samples



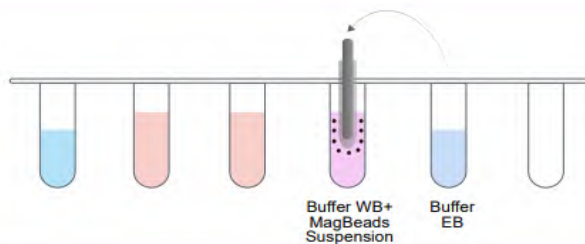
2 Bind the DNA



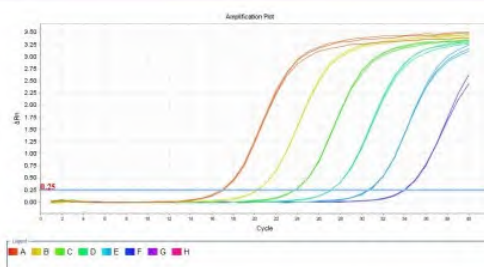
4 Elute the DNA



5 Discard the beads



6 qPCR test



*Binding Buffer: Buffer LB 400µL+CR Solution 3µL

Prepare the reagents and samples for DNA extraction by ACRO OPE-32S

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 SERC, NEC and ERC. (Optional)

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

Protocol for DNA extraction by ACRO OPE-32S

The following steps apply for ACRO OPE-32S.

1. Instrument Preparation

1.1 Wipe down the processing chamber with 75% ethanol before use.

1.2 Close the chamber door, activate the UV lamp program, and irradiate the chamber with UV light for 15 minutes.

2. Sample Digestion

2.1 Please refer to manual DNA extraction, point 1.1~1.5 on page 7.

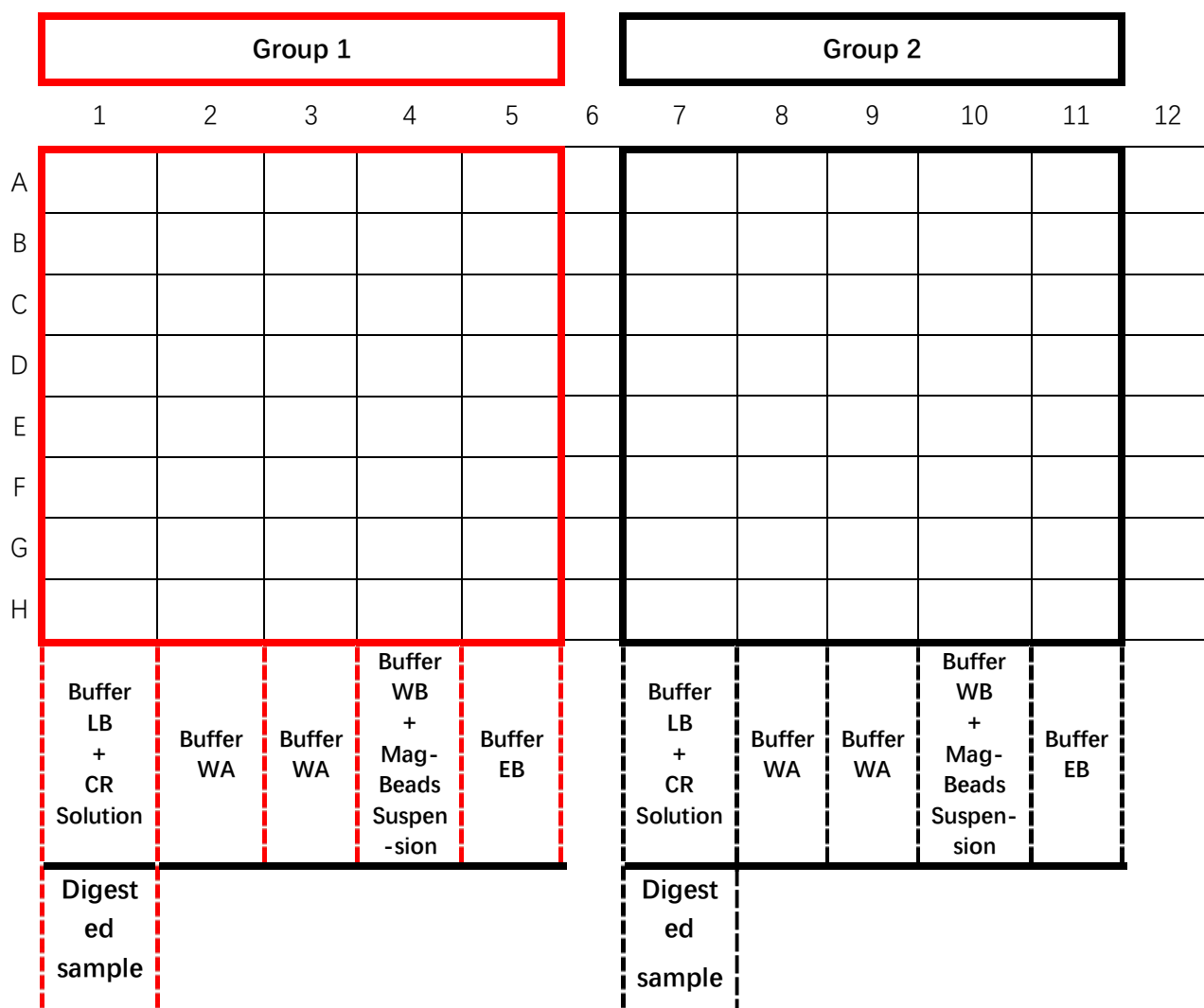
3. Reagents Dispensing

3.1 Calculate the volume of the binding buffer according to the number of the tested samples. Prepare the binding buffer in a 50 mL tube according 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 μ L	400 μ L $\times$ N
CR Solution	3 μ L	3 μ L $\times$ N
Total	403 μ L	403 μ L $\times$ N

- 3.2 Dispense the reagents to the 96 V-Bottom Deep-well Plate according to the plate layout.
- 3.3 Add 403 μ L of binding buffer into wells in columns 1 and 7.
- 3.4 Add 700 μ L of Buffer WA into wells in columns 2 and 8.
- 3.5 Add 700 μ L of Buffer WA into wells in columns 3 and 9.
- 3.6 Add 700 μ L of Buffer WB, 25 μ L of MagBeads Suspension (**Vortex it thoroughly before use.**) into wells in columns 4 and 10.
- 3.7 Add 100 μ L of Buffer EB into wells in columns 5 and 11.



96 deep-well plate layout

4. Add Samples and Run the Procedure

- 4.1 Add the digested samples to the wells in columns 1 and 7 of the plate prefilled with reagents.
- 4.2 Place the loaded plate into the Deep Well Plate Station of the instrument, insert the 8-Strip V-Bottom Tip Combs into the magnetic rods until fully seated. Close the chamber door and run program **OPA_R005**. This program is preloaded in the **ACRO automated nucleic acid extraction system OPE-32S**.

NOTE: Ensure proper placement of the deep-well plate. Verify the 8-strip V-bottom tip comb is properly engaged.

- 4.3 After program completion, the instrument will make a 'tick-tick' sound, click "finish"

on the screen. Remove the 8-Strip V-Bottom Tip Combs, and then take out the 96 V-Bottom Deep-well Plate, transfer the eluted DNA in columns 5 and 11 to new 1.5 mL low DNA-binding microcentrifuge tubes or PCR tubes.

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

- 4.4 After experiment completion, wipe the instrument processing chamber with 75% ethanol. Close the chamber door, activate the UV lamp program, and irradiate the chamber with UV light for 30 minutes.

NOTE: Maintain $\geq$ 30-minute intervals between experiments to prevent cross-contamination.

PART C: 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	Ethanol, 99.7%
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

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 SERC, 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, 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](http://www.acrobiosystems.com)).

(2) Proteinase K 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.

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-R005.bdz**. Click “**Start**”. (Download the program from our official website <https://www.acrobiosystems.cn/products/kits/dna-opa-r005>)
- 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 μ L	400 μ L $\times$ N
CR Solution	3 μ L	3 μ L $\times$ N
Total	403 μ L	403 μ L $\times$ N

- 2.6 After the sample digestion is finished, take out the Lysis plate, and add 403 μ L of binding buffer, 25 μ L 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

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°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. 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.

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°C to 30°C day and night, and the temperature should not be lower than 20°C 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°C to 8°C.
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.

What is the typical elution volume?	It is recommended that the elution volume be 100 μ L, which helps maximize the efficiency of extraction.
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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
96 V-Bottom Deep-well Plate	OPA-R013	ACROBiosystems
8-Strip V-Bottom Tip Comb	OPA-R014	ACROBiosystems