EMORY UNIVERSITY

Emory Integrated Genomics Core

Emory Integrated Core Facilities

EIGC.001_Nucleic Acid Extraction Using KingFisher Flex

Standard Operating Procedure Staff Review Page:

I have read and understand the procedure listed above.

Employee name	Date SOP review complete

Standard Operating Procedure Approval Page:

Date Implemented: 23 January 2008

Updated:	Ashima Amin **Name**	12 June 2020 **Date**
Supersedes:	Ashima Amin **Name**	11 April 2016 **Date**

Annual Review and Approval

Michael Zwick, PhD
Laboratory Director

Changes Made:
061220: 96 Well protocols added.

Changes to previous procedures:
041116: LIMS steps updated.

Note: No laboratory personnel should use this piece of equipment without proper training by the laboratory supervisor or other designated person.

Instrument Principles: The KingFisher Flex is an automated magnetic particle processor designed to work with any magnetic bead-based purification kit. For DNA extractions, biological specimens are first lysed to release DNA content. The DNA selectively binds to the paramagnetic beads leaving cellular debris and other contaminants behind. The Flex is equipped with two interchangeable magnetic heads, in 96-well and 24-well configuration, for maximum flexibility.

Specimen Types: Whole blood, buffy coat, PBMC in freezing media, packed blood cells, mouthwash buccal cells, saliva, or tissue (FFPE or fresh) samples.

Required Reagents and Consumables:

Extraction reagents:

1. For designated 24 well format protocols:
    a. KF Flex 24 well DW tip comb, Thermo Scientific, 97002610
    b. KF Flex 24 well DW plates, Thermo Scientific, 95040470
2. For designated 96 well format protocols:
    a. KF Flex 96 well DW tip comb, Thermo Scientific, 97002534
    b. KF Flex 96 well DW plates, Thermo Scientific, 95040460
    c. KF Flex 96 well shallow plates, Thermo Scientific, 97002540
3. 50 mL Falcon Tubes
4. 1.5- or 2.0-mL screw cap microcentrifuge tubes
5. 100% isopropanol
6. 100% ethanol
7. 1X TE buffer (pH 7.9-8.1), Promega, V6232
8. 1X PBS
9. RNaseA, Qiagen, 19101

Extraction Kits – Omega Biotek

Mag-Bind SQ Blood DNA Isolation Kit (cat# M6213-02) - custom	
Buffer NL	Room Temp
Buffer XL	Room Temp
Wash Buffer SPM Concentrate	Room Temp
Elution Buffer	Room Temp
Magnetic Particle Solution E	4°C
Proteinase K	4°C after reconstitution for 1 week -20°C for one month

Mag-Bind Blood & Tissue DNA HDQ 96 Isolation Kit (cat# M6399-00)	
AL Buffer	Room Temp
TL Buffer	Room Temp
VHB Buffer Concentrate	Room Temp
HDQ Binding Buffer Concentrate	Room Temp
SPM Buffer Concentrate	Room Temp
Elution Buffer	Room Temp
Mag-Bind Particles HDQ	4°C
Proteinase K Solution	Room temp for 12 months 4-8°C for long term

Mag-Bind Saliva DNA Isolation Kit (cat# M0312-EUW) - custom	
Buffer BL	Room Temp
Wash Buffer QMP Concentrate	Room Temp
Wash Buffer SPM Concentrate	Room Temp
Elution Buffer	Room Temp
Magnetic Particle Solution E	Room Temp
RNaseA	4°C
Proteinase K	4°C after reconstitution for 1 week -20°C for one month

Mag-Bind FFPE DNA KF 96 Kit (cat#* M6954-00*) - custom	
FTL Buffer	Room Temp
MB3 Buffer	Room Temp
RMP Buffer	Room Temp
DNA Wash Buffer	Room Temp
LPA	Room Temp
Proteinase K Storage Buffer	Room Temp
Elution Buffer	Room Temp
RNase A	4°C
Mag-Bind Particles CNR	4°C
Proteinase K	4°C after reconstitution for 1 week -20°C for one month

Quality Control: All samples are registered into our Laboratory Information Management System (LIMS) and evaluated for quality by EIGC staff members. All isolated DNA is quantitated by spectrophotometric methods using the Tecan Infinite M200 Pro and evaluated for quality by agarose gel electrophoresis.

> See ***EIGC.004_Quantification with Fluorescence*** for a detailed protocol on how to perform QC using the Tecan Infinite M200Pro.
>
> See ***EIGC.008_General Agarose Gel Electrophoresis*** for a detailed protocol on how to perform QC using agarose gels.

Safety: All employees are required to follow universal safety protocols when working with potentially infectious biohazardous materials. Safety procedures are outlined in the Emory University Bloodborne Pathogen Exposure Control Plan (see Employee Safety Notebook).

Extraction Recording Keeping and LIMS: The extraction date is recorded in the LIMS system as the date DNA is received. Extraction kit lot numbers are recorded in the QC report that is passed on to the client.

Procedural Steps:

Blood Extraction (10 mL) – SQ Kit 24 Well Plate Format

1. Print two LIMS labels for each sample: one for a 50 mL Falcon tube and one for the elution tube.
2. Prepare proteinase K and SPM wash buffer according to bottle or product insert.
3. Dispense 25 mL buffer NL into each labeled 50 mL Falcon tube.
4. Add 10 mL whole blood into the Falcon tube. Gently invert 15-20 times to mix.
   Note: Save the blood vacutainer tube with residual blood left as backup.
5. Centrifuge the Falcon tube at 3,000 × g for 5 min.
6. Slowly pour off the supernatant taking care not to disturb the pellet. Blot dry the tube rim on a piece of Blood Bloc.
7. Add 2.5 mL of Buffer XL to the tube and immediately vortex for 30 s or until the pellet is completely resuspended.
   Note: Lysed samples may be stored at room temperature for up to a month.
8. Scan the tube ID in a 4x6 format plate map to keep a record of where samples are in the plate.
9. Set up the following Flex 24 DW plates with indicated volumes in each well.

   Note: Ensure 30 µl proteinase K is added to the lysis plate before adding the lysate.

   a. Tip Comb (comes packaged in a DW plate; leave comb inside plate)
   b. Lysis – 2.5 mL XL Lysate + 30 µl proteinase K
   c. SPM Wash 1 – 3 mL SPM
   d. SPM Wash 2 – 2 mL SPM
   e. Elution – 1.5 mL Elution Buffer

10. From the KF Flex touch pad, start the program "**10 mL Blood NL**".
11. Load the plates when prompted. Make sure the plate orientation is correct by matching well A1 to the A1 mark on the turntable.
12. Press Start to begin extraction. Set a timer for 15 min.
13. Make a master mix of 2.5 mL 100% isopropanol and 100µl magnetic beads for each well.
14. After ~15 min, the robot will pause, and a message will appear on the touch pad screen "**+2.5 ml IPA/100µlBeads**".
15. Remove the lysate plate, add the master mix, and load it back onto the KF. Continue the program.

16. After extraction is complete, incubate the plate with tip comb in at 50°C for 1 hr (shaking is optional). Leave the plate at room temperature overnight with the tip comb in.

17. The following day run the program **"Beads Pickup"** from the KF Flex touchpad.

18. Place the elution plate on a magnetic plate stand until cleared to remove any residual beads.

19. Transfer samples to barcoded 2 mL screw-top tubes.

20. Proceed to QC Protocols.

Saliva Extraction – 24 Well Format

1. Print two LIMS labels for each sample: one for a 50 mL Falcon tube and one for the elution tube.

   Note: Each saliva sample will need to be split into two wells in the plate.

2. Prepare proteinase K, QMP and SPM wash buffers according to the product insert.
3. To a Flex 24 DW plate, add 20µl proteinase K to each well.
4. Scan the tube ID in a 4x6 format plate map to keep a record of where samples are in the plate.
5. Use a disposable transfer pipet to transfer 1.75 mL of the saliva sample to each of the two wells.

   Note: For sponges: Place a syringe with the stopper removed into a 50 mL Falcon tube. Add sponges and transfer any additional liquid with a transfer pipette into the syringe barrel. Spin at 1,000 rpm for 10 minutes. Process as above by splitting the sample into two wells.

6. Seal the plate with adhesive film. Incubate at 50°C overnight.
7. Make a master mix of 1.5 mL BL and 10µl RNaseA and add to each sample well.
8. Set up the following Flex 24 DW plates with indicated volumes in each well:
   a. Tip Comb (comes packaged in a DW plate; leave comb inside plate)
   b. Lysate - 5 mL saliva/proteinase K/BL/RNaseA
   c. QMP Wash 1 - 2.5 mL QMP
   d. SPM Wash 1 - 2 mL SPM
   e. SPM Wash 2 – 2 mL SPM
   f. Elution - 0.5 mL Elution Buffer
9. From the KF Flex touch pad, start the program "**1750ul Saliva or Blood**".
10. Load the plates when prompted. Make sure the plate orientation is correct by matching well A1 to the A1 mark on the turntable.
11. Press Start to begin extraction. Set a timer for 20 min.
12. Make a master mix of 1.7 mL 100% isopropanol and 50 µl magnetic beads for each well.
13. After ~20 min, the robot will pause, and a message will appear on the touch pad screen "**+1.7mL IPA/50µlBeads**".
14. Remove the lysate plate, add the master mix, and load it back onto the KF. Continue the program.
15. After the extraction is complete, place the elution plate on a magnetic plate stand until cleared to remove any residual beads.
16. Transfer the samples to barcoded 1.5 mL screw-top tubes.
17. Proceed with QC protocols

FFPE DNA Extraction (Using Heat) – 24 Well Format

1. Prepare DNA Wash buffer, MPW Buffer, and Proteinase K according to instructions on bottles.
2. Print labels in LIMS
3. Use 8-10 FFPE sections 5-10 µm thick and place in 1.5 ml microcentrifuge tubes.
4. Add 250 µl FTL buffer to the tube.
5. Centrifuge at maximum speed (≥14,000 × g) for 60 s.
6. Incubate at 90°C for 15 min. Mix the sample a few times by gently shaking the tube 2-3 times. Ensure the tissue sections stay submerged in the solution.
7. Incubate at room temperature for 5 min.
8. Add 20 µl Proteinase K Solution.
9. Incubate in the shaker overnight at 55°C.
10. Centrifuge at maximum speed (≥14,000 × g) for 5 min. The paraffin will form a thin layer on top of the lysate solution.
11. Transfer 200 µl cleared lysate into a 96-well plate.

    Note: Use a 1 mL pipette tip to penetrate the paraffin layer.
12. Add 3 µl RNase A to each sample and mix.
13. Incubate for 15 min at room temperature.
14. Add 10 µl LPA to each sample and mix.
15. Make a master mix of 500 µl MB4 and 30 µl Mag-Bind Particles CNR for each sample and add to plate.
16. Add the sample positions to an extraction log to keep record of where samples are in the plate.
17. Prepare the following KF Flex processing plates:

Plate Type	Content	Reagent Volume
KF 96-Deep Well	Lysate LPA MB4 Buffer Mag-Bind Particles CNR	200 µl 10 µl 500 µl 10 µl
KF 96-Deep Well	MPW Buffer	500 µl
KF 96-Deep Well	DNA Wash Buffer	800 µl
KF 96-Deep Well	DNA Wash Buffer	800 µl
KF 96 Well Shallow	Elution Buffer	100 µl
KF 96 Tips	Tips (placed in a shallow plate)	

18. From the KF Flex touch pad, start the program "**FFPE DNA**".
19. Load the reagent plates when prompted. Make sure the correct orientation of the plates by matching well A1 to the A1 mark on the turntable.
20. Press Start to begin extraction.
21. Transfer the samples to barcoded 1.5 mL screw-top tubes.
22. Proceed with QC protocols.

PBMC Extraction – HDQ 96 Well Format

1. Print labels in LIMS.
2. Thaw cells at 37°C until just thawed.
3. Pellet up to 5 × 10^6 cells by centrifugation for 2 min at 1200 × g and 4°C.
4. Remove and discard supernatant.
5. Resuspend cell pellet in 500 µl cold 1X PBS by gently pipetting.
6. Transfer 250 µl to a KF DW 96 plate.
7. Make a sample extraction log to keep a record of where samples are in the plate.
8. Make a master mix of 20 µl Proteinase K Solution and 290 µl AL Buffer for each sample and add to samples.

   Note: For best results, prepare master mix immediately before adding to samples.
9. Mix thoroughly by pipetting up and down.
10. Add 5 µl RNase A and mix thoroughly by pipetting up and down.
11. Using the lab oven, incubate at 60°C for 20 min. Vortex gently every 5 min. During incubation, set up other reagent plates according to Plate Layout table below. Seal with a foil film to prevent evaporation:

Plate Type	Content	Reagent Volume
KF 96-Deep Well	Lysate/Proteinase K/AL Buffer RNase A	565 µl
KF 96-Deep Well	VHB Buffer	500 µl
KF 96-Deep Well	VHB Buffer	500 µl
KF 96-Deep Well	SPM Buffer	500 µl
KF 96-Deep Well	Molecular grade water	500 µl
KF 96 Well Shallow	Elution Buffer	100 µl
KF 96 Tips	Tips (placed in a shallow plate)	

12. After incubation, transfer the samples to a new KF DW 96 plate. If any cellular debris is present, centrifuge at 4000 × g for 5 min to pellet debris before transferring samples.
13. Make a master mix of 400 µl HDQ Binding Buffer and 20 µl HDQ Magnetic Particles for each sample and add to samples.
14. Mix thoroughly by pipetting up and down 5-10x.
15. From the KF Flex touch pad, start the program "**Omega_M6399_DNA_HQD_96**".

16. Load the plates when prompted. Make sure the plate orientation is correct by matching well A1 to the A1 mark on the turntable.
17. Press Start to begin extraction.
18. After the extraction is complete, place the elution plate on a magnetic plate stand until cleared to remove any residual beads.
19. Transfer the samples to barcoded 1.5 mL screw-top tubes.
20. Proceed with QC protocols.

Buffy Coat Extraction – HDQ 96 Well Format

1. Print labels in LIMS
2. Thaw Buffy Coat at 37°C or room temperature.
3. Transfer 100 µl of Buffy Coat to KF DW 96 plate.
4. Bring up to 250 µl by adding 150 µl of 1X PBS buffer.
5. Make a master mix of 20 µl Proteinase K Solution and 290 µl AL Buffer for each sample and add to samples.

   IMPORTANT: prepare master mix immediately before adding to samples.

6. Mix thoroughly by pipetting up and down.
7. Make a sample extraction log to keep a record of where samples are in the plate.
8. Add 5 µl RNase A and mix thoroughly by pipetting up and down.
9. Using the lab oven, incubate at 60°C for 30 min. Vortex gently every 5 min.
10. During incubation, set up other reagent plates according to Plate Layout table below. Seal with a foil film to prevent evaporation:

Plate Type	Content	Reagent Volume
KF 96-Deep Well	Lysate/Proteinase K/AL Buffer RNase A	565 µl
KF 96-Deep Well	VHB Buffer	500 µl
KF 96-Deep Well	VHB Buffer	500 µl
KF 96-Deep Well	SPM Buffer	500 µl
KF 96-Deep Well	Molecular grade water	500 µl
KF 96 Well Shallow	Elution Buffer	100 µl
KF 96 Tips	Tips (placed in a shallow plate)	

9. Make a master mix of 400 µl HDQ Binding Buffer and 20 µl HDQ Magnetic Particles for each sample and add to samples.
10. Mix thoroughly by pipetting up and down 5-10x.
11. From the KF Flex touch pad, start the program "**Omega_M6399_DNA_HQD_96**".
12. Load the plates when prompted. Make sure the plate orientation is correct by matching well A1 to the A1 mark on the turntable.
13. Press Start to begin extraction.

14. After the extraction is complete, place the elution plate on a magnetic plate stand until cleared to remove any residual beads.
15. Transfer the samples to barcoded 1.5 mL screw-top tubes.
16. Proceed with QC protocols.

Analysis/Interpretations:

Analysis of DNA extraction by the KF Flex is done based on quantitation and assessing the quality of the extracted DNA. See EIGC.004_Quantification with Fluorescence for fluorescent quantitation protocol or EIGC.008_General Agarose Gel Electrophoresis for the agarose gel quality check protocol.

Troubleshooting:

For instrument related troubleshooting, consult the Troubleshooting Guide in the KingFisher Flex User Manual. For DNA quality troubleshooting, consult the Omega BioTek website for product specific troubleshooting guides: http://www.omegabiotek.com/ or call 1-800-832-8896.