Standard Operation Procedure

Genomic DNA Extraction From Peripheral Venous Blood and Cord Blood samples on Kingfisher Flex

How to start

Items required by the user:

1. Kingfisher Flex system
2. 100% Ethanol
3. 80% Ethanol

Protocol to refer

S No.	Sample Type	Protocol to be followed
1	Peripheral Venous Blood (PVB)	Genomic DNA extraction from PVB samples
2	PVB from Mother	Genomic DNA extraction from PVB samples
3	Cord Blood	Genomic DNA extraction from Cord Blood Samples

Sample Handling

1. A starting sample volume of 200 µL of whole blood and 200 µL of cord blood is suggested for DNA extraction.
2. Before pipetting out blood, gently invert the vacutainer containing the blood sample 2-3 times for homogenous mixing.

Preparation of working solutions

1. **Buffer CDL**: Pre-heat Buffer CDL at 70°C to dissolve the buffer precipitates.

1. Protocol for genomic DNA extraction from Peripheral Venous Blood (PVB) samples

A. Setting up the plates on the Kingfisher Flex platform

1. Ensure that the program "**KFF_WB_CB_Protocol.bdz**" is downloaded and installed in the machine.
2. Fill the appropriate volume of buffers into the deep-well plates according to the specified volumes in the below-mentioned table and keep them ready.

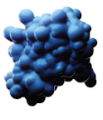
PLATE INDEX	PLATE POSITION	BUFFER	VOLUME	PLATE TYPE
Wash I Plate	2	Wash Buffer I	500 µL	V - bottom plate
Wash II Plate	3	Wash Buffer II	500 µL	V - bottom plate
Wash III Plate	4	80% Ethanol	300 µL	V - bottom plate
Elution Plate	5	Elution Buffer	100 µL	Elution plate
96-well Tip comb	6	Place a 96-well tip comb into a V - bottom plate		

B. Preparing the Sample Plate for the lysis of Peripheral Venous Blood (PVB) samples

The pre-digestion and lysis of the whole blood sample is carried out in the Sample plate. Add the following buffers in the below-mentioned order into the sample plate for lysing the blood sample.

PLATE INDEX	PLATE POSITION	BUFFER	VOLUME
Sample Plate	1	Proteinase K solution	30 µL
		Blood sample	200 µL
		Buffer BL	200 µL
		Buffer LE	30 µL
		Total	**460 µL**

(**Note**: Do not change the order of addition of the buffers into the wells of the deep-well plate)



C. Loading the plates into the Kingfisher Flex extraction platform

1. Select the installed program "**KFF_WB_CB_Protocol.bdz**" and run the program
2. Once prompted by the instrument, load the plates onto their appropriate positions on the extraction platform and start the extraction process.
3.

D. Preparing the CamBeads and Lysis-Binding Buffer Mix

1. Prepare the Cambeads and Lysis-Binding buffer in a sterile container according to the number of extractions on the 96-well plate.

PLATE Name	PLATE POSITION	LYSIS - BINDING BUFFER (per reaction)	CAMBEADS (per reaction)	TOTAL VOLUME PER WELL/ PER REACTION
Sample Plate	1	500 µL	20 µL	520 µL

   (Vortex the Cambead-Lysis-Binding buffer mixture thoroughly before adding it to the wells of the Sample plate).
2. The machine will pause after pre-digestion. Remove the Sample plate from the machine and add 520 µL of the Cambead-Lysis-Binding buffer mixture into each well of the 96 well plate.
3. Place the plate back to Slot 1 of the machine and follow the prompts on the machine to allow the extraction process to proceed.
4. After the extraction process is complete, collect the elutes from the elution plate and store the eluted DNA at -20°C.

2. Protocol for genomic DNA extraction from Cord Blood Samples

A. Setting up the plates on the Kingfisher Flex platform

1. Ensure that the program "**KFF_WB_CB_Protocol.bdz**" is downloaded and installed in the machine.
2. Fill the appropriate volume of buffers into the deep-well plates according to the specified volumes in the below-mentioned table and keep them ready.

PLATE INDEX	PLATE POSITION	BUFFER	VOLUME	PLATE TYPE
Wash I Plate	2	Wash Buffer I	500 µL	96-well deep well v bottom plate
Wash II Plate	3	Wash Buffer II	500 µL	96-well deep well v bottom plate
Wash III Plate	4	80% Ethanol	300 µL	96-well deep well v bottom plate
Elution Plate	5	Elution Buffer	100 µL	96-well elution plate
96-well Tip comb	6	Place 96-well tip comb into a standard 96-well deep well plate		

B. Preparing the Sample Plate for the lysis of the cord blood sample

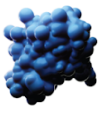
The pre-digestion of the cord blood sample is carried out in the Sample plate. Add the following buffers in the below-mentioned order into the sample plate for lysing the cord blood sample.

PLATE INDEX	PLATE POSITION	BUFFER	VOLUME
Sample Plate	1	Proteinase K solution	30 µL
		Cord Blood sample	200 µL
		Buffer CDL	200 µL
		Total	**430 µL**

(**Note**: Do not change the order of addition of the buffers into the wells of the deep-well plate)

C. Loading the plates into the Kingfisher Flex extraction platform

1. Select the installed program "**KFF_WB_CB_Protocol.bdz**" and run the program
2. Once prompted by the instrument, load the plates onto their appropriate positions on the extraction platform and start the extraction process.



D. Preparing the CamBeads and Lysis-Binding Buffer Mix

1. Prepare the Cambeads and Lysis-Binding buffer in a sterile container according to the number of extractions on the 96-well plate.

PLATE Name	PLATE POSITION	LYSIS - BINDING BUFFER (per reaction)	CAMBEADS (per reaction)	TOTAL VOLUME PER WELL/ PER REACTION
Sample Plate	1	500 µL	20 µL	520 µL

(Vortex the CamBead-Lysis-Binding buffer mixture thoroughly before adding it to the wells of the Sample plate.)

2. The machine will pause after pre-digestion. Remove the Sample plate from the machine and add 520 µL of the Cambead-Lysis-Binding buffer mixture to the lysate in each well of the 96 well plate.
3. Place the plate back to Slot 1 of the machine and follow the prompts on the machine to allow the extraction process to proceed.
4. After the extraction process is complete, collect the elutes from the elution plate and store the eluted DNA at -20°C.