Standard Operation Procedure

Genomic DNA Extraction From Dried Blood Spots on Kingfisher Flex

How to start

Items required by the user:

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

Preparation of working solutions

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

Protocol for genomic DNA extraction from Dried Blood Spots

A. Setting up the plates on the Kingfisher Flex platform

1. Ensure that the program "**KFF_DBS_Protocol_V3.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	800 µL	V - bottom plate
Wash II Plate	3	80% Ethanol	300 µL	V - bottom plate
Wash III Plate	4	80% Ethanol	300 µL	V - bottom plate
Elution Plate	5	Elution Buffer	50 µL	Elution plate
Comb Plate	6	Place a 96-well tip comb into a V - bottom plate		

B. Sample Preparation

1. Prepare **~42 sq. mm** punches from the dried blood spots on the DBS card using a metal-hand punch. The table below provides guidance on the number of punches to use for a given diameter

Spot Diameter (mm)	No. of Spots	Area (sq. mm.)
3	3	28.3

	4	37.7
	5	47.1
4.5	3	28.3
	4	37.7
	5	47.1

2. Transfer these discs to each well of the 96-well plate (**Pre-Lysis plate**) using a pair of sterile forceps

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C. On-Deck Pre-Lysis of the DBS spots

1. To the discs in each well of the 96-well **Pre-Lysis Plate,** add the buffers in the following order.

PLATE INDEX	PLATE POSITION	BUFFER	VOLUME
Pre-Lysis Plate	1	Buffer RCB	200 µL
		Proteinase K solution	30 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)

2. Select the installed program "**KFF_DBS_Protocol_V3.bdz**" and run the program.
3. The on-deck lysis will complete in **30 minutes**. Remove the Pre-Lysis plate and **carefully transfer the lysate (without disturbing the discs at the bottom of the plate) to the wells of a new 96-well deep well plate. Place this plate on position 1**. This new plate shall be referred to as **"Sample Plate".**

D. Preparing the CamBeads and the 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	250 µL	20 µL	270 µL

(**NOTE**: Vortex the CamBead-Lysis-binding buffer mixture thoroughly before adding 270 µL of the mixture to each of the lysates in the wells of the Sample plate).

2. Place the plate on Slot 1 of the machine and follow the prompts on the machine to allow the extraction process to proceed.

3. After the extraction process is complete, collect the elutes from the elution plate and store the eluted DNA at -20°C.