PROTOCOL

Covaris

truXTRAC™ DBS DNA Kit (25)

Adaptive Focused Acoustics™ (AFA) based
DNA extraction and purification from Dried Blood Spots (DBS)

INTENDED USE

The truXTRAC DBS DNA Kit is intended for use in molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

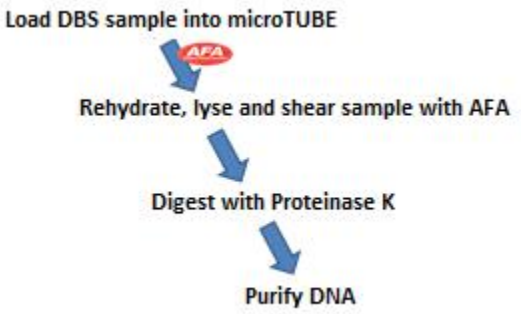
INTRODUCTION

The truXTRAC DBS DNA Kit is designed for the efficient extraction of NGS-grade DNA from Dried Blood Spot (DBS) samples with Covaris Adaptive Focused Acoustics™ (AFA) technology. Covaris AFA promotes rapid sample rehydration and cell lysis, while maintaining precisely controlled DNA shearing, resulting in high yields of DNA fragments of a predetermined size. The extracted DNA is of high quality and ideally suited for Next Generation Sequencing library construction.

This protocol is optimized for blood spots smaller than 3 mm in diameter.

PROCEDURE WORKFLOW OVERVIEW

Fragment size can be tuned by adjusting treatment time.



Please contact Covaris at Application Support (ApplicationSupport@covarisinc.com) for user support and inquires

REVISION HISTORY

Part Number	Revision	Date	Description of change
010288	A	July 2015	Initial release
010288	B	December 2015	Changes AFA settings

KIT CONTENTS

Tissue SDS Buffer	10 ml
Buffer B1	7.5 ml
Buffer B5	7 ml
Buffer BW	15 ml
Buffer BE*	7.5 ml
PK Solution	275 µl
Purification Columns	25
Collection Tubes	50
microTUBE-130 AFA Fiber Pre-Slit Screw-Cap	25

*Buffer BE Composition is 5 mM Tris HCl pH 8.5

SDS INFORMATION IS AVAILABLE AT http://covarisinc.com/resources/safety-data-sheets/

STORAGE

This kit should be stored at room temperature (18 – 25 °C) upon receipt.

SUPPLIED BY USERS

Covaris Instruments and Parts

Focused-ultrasonicator	S-Series	E220 & E210	E220 evolution
Rack/ Holder	Holder microTUBE Screw-Cap (PN 500339)	Rack 24 Place microTUBE Screw-Cap (PN 500308)	Rack E220e 4 Place microTUBE Screw-Cap (PN 500432)
Intensifier	NA	Intensifier (PN 500141)	Intensifier (PN 500141)
Accessories	Centrifuge and Heat Block microTUBE Adapters (PN 500406)		

Other supplies:

- Microcentrifuge with 11,000 x g capability
- Dry heater, such as Eppendorf ThermoMixer or similar with heat block for 1.5 or 2-ml tubes.
- Ethanol (>96%), MB Grade e.g., Thermo Scientific (PN BP2818-100)
- 1.5 mL nonstick nuclease free microfuge tubes e.g., Life Technologies (PN AM12450)

1 – PREPARATION

Focused-ultrasonicator

Set up the instrument as shown in Table 1. Wait for the water to reach temperature and to degas. For more detailed instructions on how to prepare your specific instrument, please refer to your instrument's User Manual.

Table 1 – Instrument Set-up

Instrument	Water level*	Chiller temp.	Intensifier	Plate definition**	Holder or Rack
S-Series	**15**	**18°C**	**NA**	NA	PN 500339
E220 or E210	**10**	**18°C**	**yes**	Rack 24 Place microTUBE Screw-Cap	PN 500308
E220 evolution	**10**	**18°C**	**yes**	Rack E220e 4 Place microTUBE Screw Cap	PN 500432

***Use Run side of Fill/Run scale**

** **If you do not see a plate definition on your system, please contact Covaris technical support at TechSupport@covarisinc.com.**

BUFFERS

Add ethanol to Buffer B5: Add 28 ml of ethanol (>96%) to Buffer B5 concentrate and mark label on cap. After preparation, Buffer B5 can then be stored for one year at room temperature.

Check Buffer B1 and Tissue SDS Buffer: A white precipitate may form during storage. Dissolve any precipitates before use by incubating the bottles at 50–70°C.

2 – DNA Extraction and Shearing

1. Generate DBS punches of 3-mm or less in diameter from the dried blood spots. Up to 3 DBS punches can be added to one microTUBE-130.

2. Remove the cap from the microTUBE-130 and use tweezers to add the DBS punch(es) to the bottom of the tube. Screw the cap back onto the tube.

3. Generate the Processing Buffer as shown in Table 2.

Table 2 – Processing Buffer

# Samples	Tissue SDS Buffer	Proteinase K
8	**880** µl	**88** µl
n	**n * 110** µl	**n * 11** µl

4. Add 110 µl Processing Buffer into each microTUBE. The buffer can be added without unscrewing the tube by inserting the pipet tip through the septum in the screw-cap.

5. Process the sample using the settings provided in Table 3 to simultaneously extract and shear the DNA. Treatment time will determine the size of the DNA fragments. We recommend running a time course to determine the best treatment time for the desired fragment size, using times given in Table 4 as a guideline.

Table 3 – DNA Extraction and Shear Settings

System	Duty Factor	Peak Incident Power	Cycles per burst
S220, E220 or E220 evolution	**10%**	**175 Watts**	**200**
S2 or E210	**10%**	**5 (Intensity)**	**200**

Table 4 – Treatment Time Guideline

Treatment Time	DNA Fragment Size
130 seconds	**500 bp**
240 seconds	**300 bp**
480 seconds	**200 bp**

6. Insert the required number of Heat Block microTUBE Adapters into a heat block and set the temperature to 56°C.

7. Once the heat block has reached 56°C, load the processed microTUBEs and incubate for 1 hour.

8. Insert the required number of Heat Block microTUBE Adapters into a centrifuge, load the microTUBEs, and centrifuge at 10,000 x g for 1 min.

9. Transfer each sample into a new 1.5 ml microfuge tube (not provided). The sample can be recovered through the septum or by removing the Screw-Cap.

10. Proceed to Section 3 – **DNA Purification**.

3 – DNA PURIFICATION

Preparations

Set heat block to 70°C and preheat Buffer BE in a 1.5 mL microfuge tube.

Required volume = number of samples x 50 µl x 1.1

Procedure

1. Add 140 µl Buffer B1 to each sample and vortex thoroughly.

2. Add 160 µl ethanol (>96%) to each sample and vortex thoroughly.

3. Briefly centrifuge samples to collect liquid at the bottom of the tube.

4. For each sample, place a purification column into a collection tube.

5. Use a pipette to transfer each sample to a column.

6. Spin the assembly at 11,000 x g for 1 minute.

7. Discard the flow-through and place each column back into the original collection tube.

8. **1st wash**: Add 500 µl Buffer BW. Spin the assembly at 11,000 x g for 1 minute.

9. Discard the flow-through and place each column back into the original collection tube.

10. **2nd wash**: Add 600 µl Buffer B5. Spin the assembly at 11,000 x g for 1 minute.

11. Discard the flow-through and place each column into a **new** collection tube.

12. **Dry column**: Spin the assembly at 11,000 x g for 1 minute.

13. **Elute DNA**: Place each column into a new 1.5 ml microfuge tube (not provided) and add 50 µl pre-warmed Buffer BE (70 °C) to the center of the column. Incubate at room temperature for 3 minutes. Spin the assembly at 11,000 x g for 1 minute.

    DNA is eluted in 50 µl Buffer BE. Elution in 100 ul instead of 50 ul will increase DNA yield by about 10-15%.