PROTOCOL

Covaris

truCOLLECT™-RUO DNA Extraction & Purification Kit – Column (10)

Adaptive Focused Acoustics™ (AFA) -based DNA extraction
& column-based purification
For Research Use Only
Not for use in diagnostic procedures

Product PN 520236

REVISION HISTORY

Revision	Date	Description of change
A	3/17	As released
B	8/18	Update buffer volumes provided in the kit

Contents

Intended Use

The truCOLLECT™-RUO DNA Extraction & Purification Kit is intended for use in life science applications, such as molecular biology. This kit is designed to be used in conjunction with the truCOLLECT-RUO Specimen Transport kit (PN 520184) to extract and purify DNA from dry stabilized blood specimens.

This Research Use Only product is not intended for the diagnosis, prevention, or treatment of a disease.

Introduction

DNA extraction from blood is the method of choice for researchers, however, the logistics of collection, stabilization, and long-term storage, as well as DNA extraction from dried blood specimens remains difficult to adapt for use in downstream NGS-based analysis, due to inherently low DNA yields. The truCOLLECT-RUO DNA Extraction & Purification Kit is specifically designed for the truCOLLECT-RUO Specimen Transport Kit for laboratory-based extraction and purification of DNA from dry-stabilized whole blood. DNA recovery from such dry-stabilized blood samples is performed using Covaris Adaptive Focused Acoustics (AFA™), which ensures rapid rehydration and detachment of blood cells from the truCOLLECT-RUO swab. Furthermore, optimized DNA extraction buffers in combination with AFA ensures the efficient isolation and subsequent column-based purification of high quality, molecular biology grade DNA. The yield of genomic DNA is dependent on white blood cell count and is generally in the range of 5 to 15 ng per microliter of blood.

Kit Contents

Item	Amount per sample	Amount included per kit (10 samples)
microTUBE-130 AFA processing tube	1	10
Purification column	1	10
Collection tube	3	30
AFA Conditioning Buffer	235 µl	5 ml
Proteinase K (PK)	20 µl	325 µl
B3 Buffer	200 µl	4.5 ml
BW Buffer	500 µl	6 ml
B5 Buffer (Concentrate)	600 µl*	2 ml
BE Buffer	100 µl	3 ml
Product Insert	n/a	1

*amount of prepared B5 from concentrate

STORAGE

Store the Proteinase K solution at 2-8 °C.

Store all other kit components at room temperature.

SUPPLIED BY USERS

Required Reagents not provided within this Kit:

Item	Amount per sample	Amount needed per kit (10 samples)
truCOLLECT™-RUO dry-stabilized sample (PN 520184)	1	10
96-100% molecular biology grade ethanol* for samples	210 µl	2.1 ml
96-100% molecular biology grade ethanol* for B5 Buffer	n/a	8 ml

*Ethanol, absolute alcohol, 200 proof (e.g., AmericanBio, PN AB00515)

Equipment Required:

1. 1.5 ml Nuclease free Microfuge Tubes (2 per sample) (e.g., Eppendorf Safe-Lock Tubes, PN 022363212)
2. Benchtop microcentrifuge (11,000 x g capability)
3. Dry block heater for 1.5 mL tubes, capable of heating to 70°C (e.g., Eppendorf ThermoMixer C, PN 2231000269)
4. Pipettes and Pipette Tips (suggested: 20, 200 and 1000 µl)
5. Benchtop vortex

FOCUSED-ULTRASONICATOR SUPPLIES AND SET UP

Instrument	Water level*	Chiller Set Point	Water Temp.	Intensifier PN500141	Holder or Rack	Plate definition**
M220	**NA**	**NA**	**20°C**	**NA**	PN500414 & Insert XTU PN500489 **(***)**	NA
E220	**6**	**18°C**	**20°C**	**Yes**	Rack 24 Place microTUBE Screw-Cap PN 500308	Rack 24 Place microTUBE-130 Screw-Cap +15mm offset.plt
E220 evolution	**6**	**18°C**	**20°C**	**Yes**	Rack E220e 4 Place microTUBE Screw Cap PN500432	500432 E220e 4 microTUBE-130 Screw Cap 0.18mm offset.plt
LE-Series	**10**	**18°C**	**20°C**	**NA**	Rack-XT 24 Place microTUBE Screw-Cap PN500388	500388 Rack-XT 24 microTUBE Screw-Cap +15mm offset.plt

* 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

*** Holder PN500358, although discontinued, can also be used. This holder does not require an insert.

1. **E or LE-Series Focused-ultrasonicators:**
   Set up the instrument as shown in table. Wait for the water to reach temperature and to degas.
2. **M220 Focused-ultrasonicators**:
   Put the Holder PN500414 and the Insert PN500422 (or the discontinued Holder PN500348 without insert) in place and fill the water bath until the water reaches the top of the holder. Allow system temperature to reach 20°C.

For detailed instructions on how to prepare your instrument please refer to the respective User Manual.

EXTRACTION AND PURIFICATION PROCEDURE

Before starting:

- Set a heat block to 70°C.
- Preheat the required amount of **BE Buffer** to 70°C in 1.5ml microcentrifuge tube(s). (Number of samples * 100 * 1.5 = total volume in µl to preheat)
- Prepare **B5 Buffer** by adding 8 ml of 96-100% ethanol. Mix by inverting bottle at least 5 times. Note: ethanol reconstituted **B5 Buffer** can be stored for up to one year. However, dispense events (opening and closing bottle) should be kept to a minimum to avoid evaporation of the alcohol.

1. Add 120 µl of **AFA Conditioning Buffer** to the appropriate number of microTUBE-130 AFA processing tubes.
2. Open the truCOLLECT-RUO desiccant/storage container and remove the truCOLLECT-RUO cap/swab.
3. Carefully insert the swab into a microTUBE. Avoid spilling Conditioning Buffer. Seal the microTUBE-130 by turning the cap.
4. Process the samples using AFA treatment:

Focused ultrasonicator:	M220	E220/E220e	LE220
PIP	25 W	30 W	275 W
Duty Factor	25%	25%	25%
Cycles per Burst	1,000	1,000	1,000
Treatment Time	90 sec	90 sec	90 sec

5. Following AFA treatment, carefully unscrew and remove the cap/swab. Slowly remove 90 µl of the lysate and transfer to a 1.5 ml microcentrifuge tube.
6. Add 20 µl of **Proteinase K** solution to the microcentrifuge tube containing the lysate.
7. Add an additional 115 µl* of **Conditioning Buffer**.

*Note: the total volume should be 225 µl. Adjust the volume of Conditioning Buffer if necessary.

8. Add 200 µl of **B3 Buffer** and mix by vortexing 15 seconds. Quick spin the tube for 1-2 seconds, if desired.
9. Incubate samples at 70°C for 15 minutes.
10. Add 210 µl of **ethanol** (96-100%) and mix by vortexing 15 seconds. Quick spin the tube for 1-2 seconds, if desired.
11. Assemble a Purification Column on top of a Collection Tube.
12. Load the entire sample onto the Purification Column.

13. Centrifuge the Column/Tube assembly for 1 minute at 11,000 x g. Discard the flow-through.
14. Place the Purification Column in a new Collection Tube.
15. **1st Wash:** Add **500 µl of BW Buffer**.

    Centrifuge 1 minute at 11,000 x g. Discard the flow-through.
16. Place the Purification Column into a new Collection Tube.
17. **2nd Wash:** Add **600 µl of prepared B5 Buffer.**

    Centrifuge 1 minute at 11,000 x g. Discard the flow-through.
18. Place the Purification Column back into the same Collection Tube and centrifuge 1 minute at 11,000 x g to remove residual ethanol.
19. Place the Purification Column into a new 1.5 ml microcentrifuge tube.
20. **Elute DNA:** Dispense 100 µl of **BE Buffer** (preheated to 70°C) directly into center of the Purification Column membrane.
21. Incubate at room temperature for 1 minute.
22. Centrifuge 1 minute at 11,000 x g to collect purified DNA sample.