

truCOLLECT™-ADK
(Application Development Kit)
PN 520245

Biological Specimen Collection,
Dry-Stabilization, Transport and Storage;

AFA Extraction Tube (130 µl)

For Research Use Only
Not for use in diagnostic procedures



UNIVERSAL PRECAUTIONS

Universal Precautions should be followed on all specimen samples, regardless of whether a sample is known to contain an infectious agent. Laboratories handling specimen samples are advised to comply with applicable parts of the following governmental and clinical standards, or their equivalent in the country of use:

- Centers for Disease Control (CDC), Universal Precautions for Prevention of Transmission of HIV and Other Bloodborne Infections, published 1987, updated 1996
- Clinical and Laboratory Standards Institute (CLSI), GP17-A3 Clinical Laboratory Safety; Approved Guideline - Third Edition, published 2012, ISBN 1-56238-797-9
- Clinical and Laboratory Standards Institute (CLSI), M29-A4 Protection of Laboratory Workers from Occupationally Acquired Infections; Approved Guideline, Fourth Edition, published 2014, ISBN 1-56238-961-0
- Occupational Safety and Health Administration (OSHA), 29 CFR 1910.1030 Bloodborne Pathogens
- International Standards Organization (ISO) 15190:2003, Medical Laboratories – Requirements for Safety

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Information subject to change without notice

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Product covered by Patent No. US 6,719,449, 6,948,843, and other applications

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REVISION HISTORY

Revision	Date	Description of change
A	3/17	As released
B	6/17	Addition of the E and LE settings

INTENDED USE

The truCOLLECT™-ADK (Application Development Kit) is intended for use as a development tool in life science applications, such as molecular biology and forensics. truCOLLECT-ADK is a Research Use Only (RUO) product and is not intended for diagnosis, prevention, or treatment of a disease. Specifically, truCOLLECT-ADK is not designed nor is it intended for direct touch specimen collection from human subjects (e.g., blood obtained from finger stick, buccal swab etc.).

truCOLLECT-ADK provides tools and a general road map to researchers for collecting, storing and transporting specimens of interest in a controlled humidity-controlled system. It also enables development of rapid Adaptive Focused Acoustics™ (AFA)-based rehydration of specimens and extraction of biomolecules using the microTUBE-130 provided in the kit.

Examples of extraction protocols are provided in the truCOLLECT-RUO DNA Extraction and Purification kit (PN 520236). With the truCOLLECT-ADK, users may develop their specific sample transport and extraction protocols based on the enabling AFA (Adaptive Focused Acoustics) technology. This includes choice of extraction buffers and downstream purification/enrichment protocols.

INTRODUCTION

truCOLLECT-ADK (Application Development kit) is specifically designed for collecting, storing and transporting valuable biological samples in a controlled desiccation system. As preservation and integrity of biological specimens may be of high importance for downstream applications such as high sensitivity analytical testing, a convenient collection, and controlled desiccation/preservation and storage/transport system, such as the truCOLLECT-ADK, is a prime requirement.

For example, analysis of whole blood specimens is a fundamental, high value technique in life science research. While freshly collected specimens are desired, the logistics of collection, stabilization, and long-term storage of fresh blood are problematic and expensive. An alternative to fresh blood is a dried blood specimen collection card technique (e.g., Dried Blood Spot). Dried blood cards, while having stability benefits and lower cost, were not designed for advanced analytical molecular biology techniques. For example, DNA extraction from conventional dried blood specimens remains difficult to standardize and integrate into NGS-workflows.

In contrast, the truCOLLECT-ADK system allows collection of specimens onto an inert swab matrix. The swab is attached to a 2D barcoded microTUBE-130 compatible cap allowing sample ID and tracking. After loading the swab, the cap/swab is immediately inserted into a desiccant/storage container, containing an immobilized desiccant and then it is sealed. This container enables consistent environment-independent, dry-stabilization of the specimen. It minimizes sample-to-sample carry-over, user error, and contamination due to environmental exposure. In accordance with standard shipping regulations, the sealed container can be shipped using standard shipping methods at ambient temperature.

In an appropriately equipped laboratory, molecules of interest (e.g., DNA, RNA, protein, metabolites etc.) can be recovered from such dry-stabilized biological samples by applying Covaris Adaptive Focused Acoustics (AFA™). AFA-energetics™ ensures rapid rehydration and detachment of the specimen from the truCOLLECT swab. The appropriate biomolecule extraction buffer may be

optimized together with AFA-energetics to enable efficient extraction and downstream isolation and purification of high quality analyte.

This Application Development Kit was developed to enable researchers to develop appropriate sample preparation protocols for their specific analytical needs and analysis systems. truCOLLECT-ADK Application Development kit is not intended for diagnostic use.

KIT CONTENTS

Item	Amount per sample	Amount included per ADK kit (10 samples)
truCOLLECT-RUO cap/swab with barcode	1	10
truCOLLECT desiccant/storage container	1	10
microTUBE-130 Screw-Cap AFA processing tube	1	10
0.1 ml PCR tube	1	10
Tamper evident indicator	1	12
Product Insert	n/a	1

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:

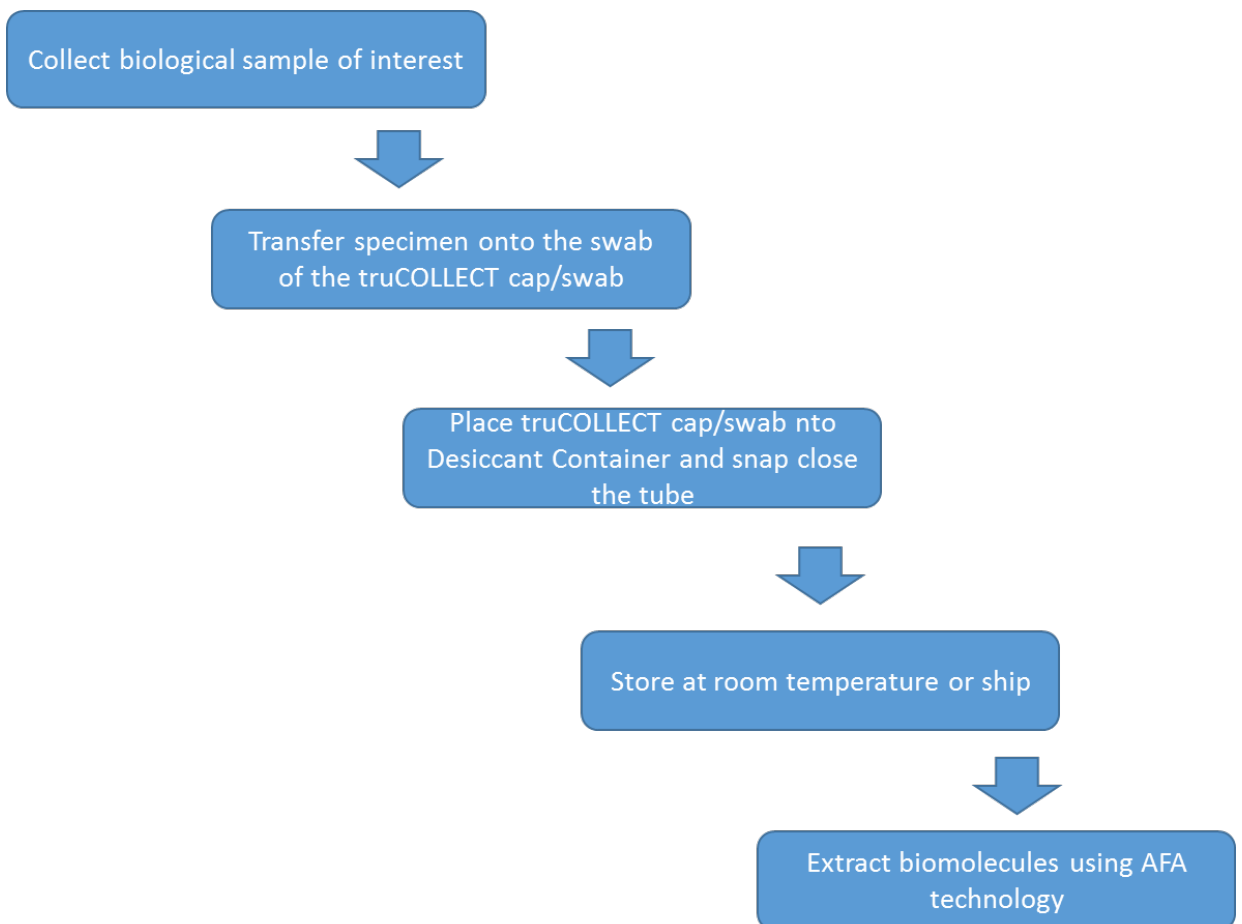
Set up the instrument as shown in table. Wait for the water to reach temperature and to degas.

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.

TRUCOLLECT–ADK APPLICATION DEVELOPMENT WORKFLOW



SAMPLE COLLECTION, DRY STABILIZATION, TRANSPORT AND STORAGE

This sample collection, dry-stabilization and storage protocol is intended for biological specimens. Examples of such specimens are blood, plasma, saliva, sputum, surface swabs, produce and meat samples, and environmental samples such as pollen.

Human specimens must not be collected by direct touch. Human samples should be collected into a primary container following appropriate procedures. For example, whole blood specimens may be collected into EDTA Blood Collection Tubes or blood collection devices such as the MiniCollect EDTA system (Greiner Bio-One). Following collection, the specimen can then be transferred from the primary collection device onto truCOLLECT-RUO swab as described in the procedure below.

Specimens can also be transferred by swabbing a surface or dipping into a specimen such as meat or plant material. Such collection is dependent on the specimen and the intended analyte to be extracted. An analyte-specific extraction method must be developed and optimized accordingly.

WARNING: As biological specimens are potentially a biohazard, Good Laboratory Practices must be followed. Appropriately equipped laboratory, trained personnel, and supplies (such as gloves, glasses, and clothing) are required to handle lab materials safely.

Example of collecting aqueous biological specimen such as blood, saliva, plasma etc.:

1. Identify the type of the specimen and the date of specimen transfer on the label of the desiccant/storage container.
2. Transfer up to 100 μ l of the sample from a primary collection device to a 0.1 ml PCR tube, using a precision pipette or transfer pipette.
3. Open the desiccant/storage container by gently pressing upwards on the tab on the cap attached to the container.
4. Carefully remove the truCOLLECT-RUO cap/swab assembly as shown in Fig. 1

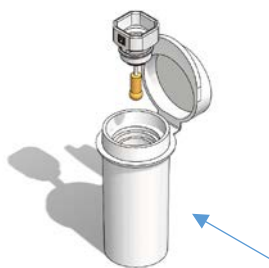


Figure 1: Opened truCOLLECT

desiccant/storage container

5. If desired, scan and note the 2D barcode number on the side of the cap/swab assembly as shown in Fig. 2.

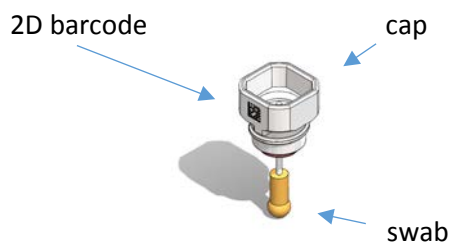


Figure 2: cap/swab assembly

CAUTION: Do not touch the truCOLLECT-RUO swab area

6. Fully immerse the swab portion of the truCOLLECT-RUO cap/swab into the 0.1 ml PCR tube containing the aliquoted specimen to collect the sample. For aqueous specimens, wait 5 seconds to make sure that the sample has wicked into the swab. Alternatively, use a pipette to apply a nominal volume of sample (e.g., whole blood capacity is < 35 μ l) directly to the swab.
7. After sample application, verify that no liquid is dripping from the swab and, being careful to avoid touching the walls of the container, immediately insert the cap/swab into the desiccant/storage container.
8. Carefully snap close the desiccant/storage container cap.

CAUTION: The desiccant/storage container needs to be firmly closed to ensure sample dry stabilization.

9. If needed, apply the tamper evident indicator tape to the desiccant/storage container by centering and wrapping the label over the cap and affixing to opposite sides of the container, careful not to cover the sample information on the label.
10. Samples may be stored at room temperature until processing.
11. If shipping is required, dry the sample overnight in the desiccant/storage container.

Always comply with current shipping regulations:

For domestic shipments in the United States, please follow the USPS Packaging Instructions for nonregulated infectious materials (USPS Packaging Instructions 6G):

http://pe.usps.com/text/pub52/pub52apxc_023.htm#ep1000450

For international shipments, please follow http://pe.usps.com/text/pub52/pub52c3_024.htm, and refer to section 346.325 Nonregulated Materials, which identifies the packaging requirements that must be met for international mail.

EXTRACTION AND PURIFICATION PROCEDURE USING AFA TECHNOLOGY

The Application Development Kit contains Covaris AFA processing vessels to enable the development of protocols based on the active extraction of biomolecules from truCOLLECT-RUO dry stabilized samples. The specimen collection, dry stabilization, transport, and storage may be from numerous, decentralized locations; however, the AFA-based extraction requires appropriate laboratory environment, specialized equipment, and trained personnel to process potentially biohazardous material.

Typically, protocols for extraction and protocols for purification of biomolecules are compatible AFA-based extraction. The Application Development Kit empowers the researcher to develop individualized protocols, while utilizing the unique benefits of the truCOLLECT-ADK system.

A sample specimen in the truCOLLECT-ADK system is desiccated. The Covaris AFA-energetics rapidly rehydrates, lyses, extracts biomolecules in an isothermal environment between 7° C and 30° C, depending on specimen type. In addition, the mechanical aspect of the AFA-energetics enables freedom of selection of appropriate extraction buffers. For example, DNA extraction from blood samples requires proteinase K compatible buffer conditions, while protein extraction is strongly dependent on the choice of buffer pH, solvent, and appropriate surfactant/detergent.

Depending on the sample specimen type and the type of biomolecule to extract, different Extraction Buffer choices and AFA-conditions may be evaluated.

A typical AFA-based extraction process is explained below:

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

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

NOTE: AFA treatment should not exceed 200W.

5. Following AFA treatment, carefully unscrew and remove the cap/swab. Slowly remove 90 µl of the lysate.
6. Transfer to a clean appropriate container and process lysate as needed (e.g., purification etc.).