



## **E Lysis Buffer**

**(Cat #10802)**

### **Instruction for manual protocol**

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## E Lysis Buffer

## Introduction

### 1. Description

Differential extraction (DE) is the most common method for processing sexual assault samples, allowing for the simultaneous recovery of sperm and epithelial cells from swab or soiled fabric pieces with the separation of sperm cells from epithelial cell DNA by exploiting the differences in the cell membrane susceptibility to detergents. DE uses proteinase K (PK) and an anionic detergent in the E-Lysis Buffer to selectively lyse the epithelial cells. The sperm cells are pelleted by centrifugation, and the supernatant which contains non-semen DNA is removed. Crime Lysis Buffer, and the reducing agent dithiothreitol (DTT), is added to the sperm pellet to lyse the sperm cells, releasing the male DNA. Both fractions are then used for further downstream analyses in an effort to obtain STR profiles of both the perpetrator and the victim.

The E-lysis procedure coupled with the Crime Prep Adem-Kit was developed to purify specially spermatozoid DNA mixed with epithelial DNA (or non sperm cell DNA).

### 2. Product Component and storage conditions

Item	E-Lysis Buffer
<b>Cat No.</b>	10802
<b>Package size</b>	1 x 48 samples
<b>E-Lysis Buffer</b>	22mL
<b>Proteinase K (10mg/ml)</b>	440µL

E Lysis Buffer (#10802)	
Reagents	Storage condition
<b>E Lysis Buffer</b>	+2-8°C
<b>Proteinase K (10mg/ml)</b>	+2-8°C

Storage conditions: The kits are shipped at room temperature.

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**NOTE 1!** Properly stored Kits are guaranteed until the expiry date. Note that shipping is realized at room temperature and will not affect stability. All components of the kit have been prepared under nucleases free conditions and have been thoroughly tested to ensure optimal performance.

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### **NOTE 2! Storage conditions**

The E Lysis Buffer can be stored at room temperature. The Proteinase K has to be absolutely stored at +2-8°C.

For convenience, you can store the whole kit at +2-8°C. In this case, before using the kit, it is recommended to take out the reagents in advance and check if there are any precipitates. If the E-Lysis Buffer presents precipitates place it at room temperature and eventually put it at +37°C.

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### ***3. Equipment and reagents to be supplied by the user***

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. To avoid contamination of your sample, wear face mask.

#### **Reagents:**

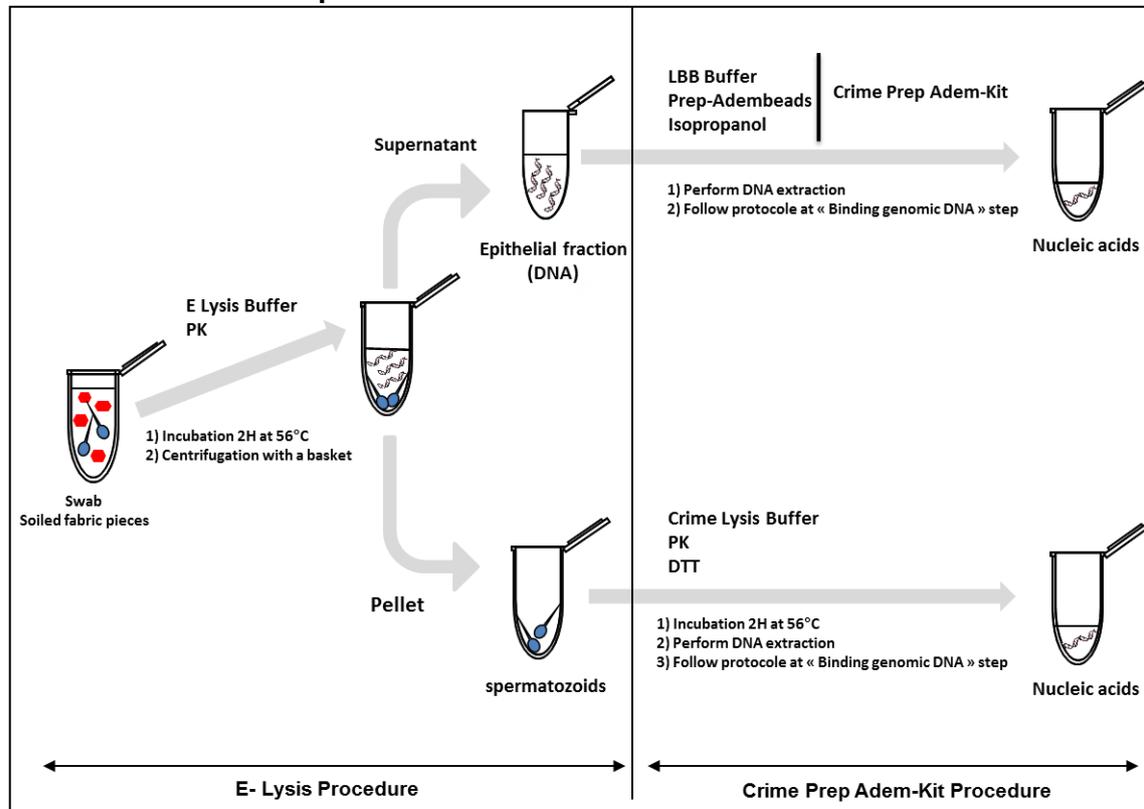
- PBS 1X or physiological water

#### **Materials:**

- 56°C heat block, thermomixer or water bath
- Microtubes
- Baskets for microtubes
- Centrifuge for microtubes

## Differential Cell Lysis Protocol

### Differential extraction protocol:



### 1. Epithelial cells lysis

1. Place the sample in a microtube (break the rod of the swab in order to be able to close the microtube).
2. Add **450µL of ELysis Buffer** to the microtube containing the sample.
3. Add **9µL of Proteinase K** to the microtube containing the sample.
4. Close the tube, homogenize and place it in a thermomixer, then incubate at +56°C and 1000rpm for 2 hours.

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**NOTE!** It is recommended to prepare a fresh premixed lysis solution by combining 450 $\mu$ L of E-Lysis Buffer and 9 $\mu$ L of Proteinase K per test.

E Lysis Buffer	450 $\mu$ L	] X number of extractions
Proteinase K	9 $\mu$ L	

In this case, add 459 $\mu$ L of premixed lysis solution to the microtube containing the sample.

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**IMPORTANT!** You can use a heat block or a water bath instead of a thermomixer. For effective recovery, make sure that the sample is immersed by the lysis solution during incubation.

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5. After epithelial cell lysis, remove the sample, place it in a basket and dispose the basket (containing the sample) in the microtube (containing the lysat).
6. Centrifuge 5 minutes at 12.000rpm.
7. Discard the basket with the sample.
8. Transfer carefully **the supernatant** containing free epithelial cell DNA in a new microtube (it is absolutely necessary not to disturb the pellet containing the spermatozoids). Keep the microtube for the following spermatozoid lysis.
9. Perform epithelial DNA extraction with **400 $\mu$ l of supernatant (epithelial fraction)**. Refer to Crime Prep Adem-Kit Instruction (start the protocol at the “Binding genomic DNA” step).

## **2. Spermatozoid cells recovery**

1. Resuspend the spermatozoid pellet with 1ml of PBS 1X or physiological water, vortex briefly and centrifuge 5 minutes at 12.000rpm.

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2. Discard carefully the supernatant without disturb the pellet.
3. Wash spermatozoid pellet one more time with 1ml of PBS 1X or physiological water, vortex briefly and centrifuge 5 minutes at 12.000rpm.
4. Discard carefully the supernatant without disturb the pellet.
5. Perform spermatozoid lysis and DNA extraction with the Crime Prep Adem-Kit. **Refer to Crime Prep Adem-Kit Instruction.**

## Warranty

This product is only for use in research. The purchaser is responsible to validate the performance of this product for any particular use, and to use the product in compliance with any applicable regulations. The products are warranted to the original purchaser only to conform to the quality and contents stated on the vial and outer labels for duration of the stated shelf life. Ademtech's obligation and the purchaser's exclusive remedy under this warranty is limited either to replacement, at Ademtech's expense, of any products which shall be defective in manufacture, and which shall be returned to Ademtech, transportation prepaid, or at Ademtech's option, refund of the purchase price. Claims for merchandise damaged in transit must be submitted to the carrier.

## Symbols

 **Reference Number**

 **Symbol for batch code/lot number.**

The symbol is accompanied by the manufacturer's batch code

 **Manufacturer**

This symbol is accompanied by the name and address of the manufacturer.

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### Expiration Date

This symbol is accompanied by a date to indicate that the device should not be used after the end of the year, month or day shown.



### Sufficient For

The number of items for which the contents of the pack is sufficient appears adjacent to the symbol.



### Temperature Limitation / Temperature Range

Symbol for temperature limitation or temperature range. Both upper and lower limits are indicated adjacent to horizontal lines.

## Ordering Information

- **Ademtech Kits**

CAT NO.	PRODUCT	PACKAGE SIZE
06213	Crime Prep Adem-Kit	1 x 96
06212	Crime Prep Adem-Kit AutoMag Solution (prefiled reagents plate)	48 (4X12)
06140	Smart D-N-Adem-Kit for Profiling	1 x 100
10801	Bone Lysis Buffer	48

- **Instrument and consumables**

CAT NO.	PRODUCT	PACKAGE SIZE
21101	AutoMag Instrument	Each
21102	Microtiter Deepwell 96 plate	50 pcs
21103	12-tip comb for 96 Deepwell plate	50 pcs
21104	Elution strip	40 pcs