Crime Prep Adem-Kit

(Cat #06211, #06213)

Instruction for manual protocol

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Introduction

1. Description

The Crime Prep Adem-Kit is specially designed for optimal DNA extraction from forensic casework samples. The Crime Prep Adem-Kit was developed to improve DNA extraction efficiency from a broad panel of sample types. These samples include blood stains, hair, cigarette butts, tissues samples and “touch” DNA samples regularly encountered in forensic DNA analysis. This instruction manual also exists for automation protocol.

2. Product Component and storage conditions

Kit Content: Each Crime Prep Adem-Kit contains sufficient reagent to perform 96 samples using the following standard protocol.

Item	Crime Prep Adem-Kit
Cat No.	06213
Package size	1 x 96 samples
Crime Lysis Buffer	40mL
Proteinase K (10mg/ml)	5mL
LB Buffer	35 mL
Prep-Adembeads	4X410µL
Washing Buffer I	24mL
Washing Buffer II	17mL
Elution Buffer	8 mL

Crime Prep Adem-Kit (#06213)	
Reagents	**Storage condition**
Crime Lysis Buffer	+2-8°C
Proteinase K (10mg/ml)	+2-8°C
LB Buffer	+2-8°C
Prep-Adembeads	+2-8°C
Washing Buffer I	+2-8°C
Washing Buffer II	+2-8°C
Elution Buffer	+2-8°C

Storage conditions: The kits are shipped at room temperature.

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.

NOTE 2! Storage conditions

All reagents in the kit can be stored at room temperature, except for the Proteinase K which has to be absolutely stored at +2-8°C. This will not affect the stability.

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 Buffers present precipitates place them at room temperature and eventually put them at +37°C.

IMPORTANT! Do not freeze the magnetic particles.

3. Equipment and reagents to be supplied by the user

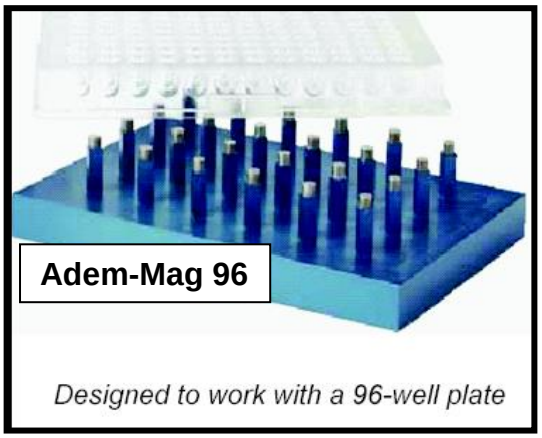
When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. To avoid contamination of your sample, wear face mask

Reagents:

- 3M Dithiothreitol (DTT)
- Isopropanol
- 96-100% ethanol
- 70% ethanol

Materials:

- 56°C heat block, thermomixer or water bath
- Microtubes or 96 Deepwell plates
- **Adem-Mag MODULO** (Cat.# 20105, # 20108)
  or **Adem-Mag 96** (Cat.# 20106)



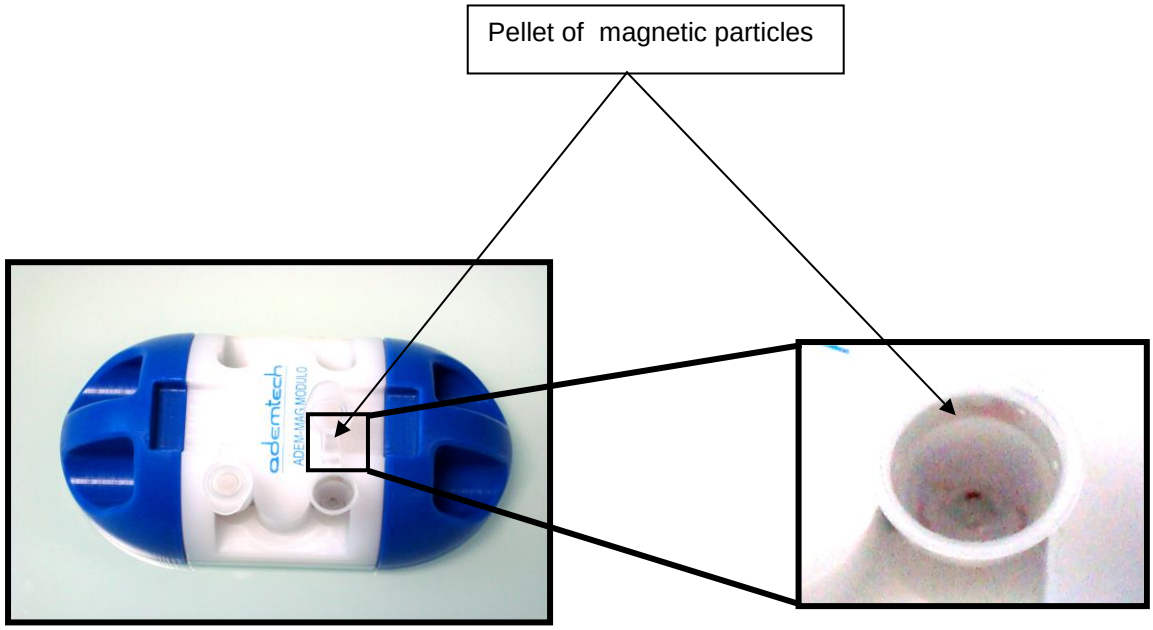
Crime Prep Adem-Kit Protocol

1. *Prep-Adembeads Guidelines*

- Before using Prep-Adembeads, thoroughly flick / vortex the bottle to completely resuspend the magnetic particles.

- During separation steps, let the microtubes containing magnetic particles on the magnet at least 5 minutes. The magnetic particles pellet is oriented toward the magnet at the back of the microtubes.

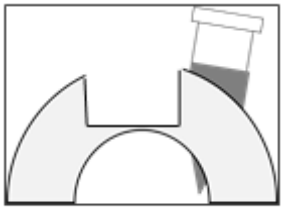
- When removing the liquid phase, pipette off carefully, do not aspirate magnetic particles or disturb the magnetic pellet



2. *Magnetic Stand Guidelines*

- Place magnetic base away from metal objects/magnetic media.

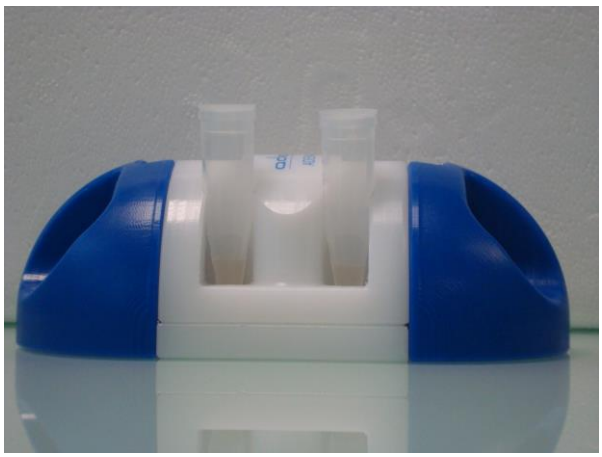
- Insert the microtubes into the sample holder in the correct position.

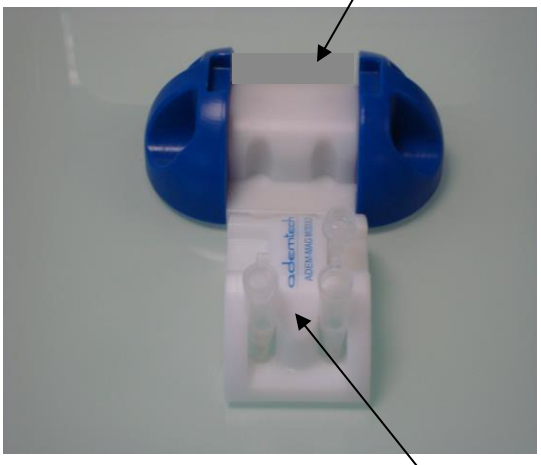


Correct position

- Insert sample holder into magnetic base. To help optimize magnetic pellet formation ensure that the magnetic stand is correctly assembled before performing washing and elution step.

- The sample holder can be quickly removed from the magnetic base to resuspend the beads





3. *Crime Prep Adem-Kit Protocol*

The standard protocol is appropriate for all kind of sample types, such as samples on solid support, liquid samples or differential extraction samples. All protocols involve a Proteinase K treatment, which is required to maximize recovery and yield from a variety of sample types, including small amounts of sample on a solid matrix, such as a swab or fabric. DNA purified using a Proteinase K treatment generally exhibits better locus-to-locus balance in downstream STR analysis.

3.1. Reagents preparation

 **WARNING ! CHEMICAL HAZARD. LB Buffer** in contact with acids or bleach liberates toxic gazes. Harmful if inhaled, absorbed through the skin, and swallowed. Cause eye, skin, and respiratory tract irritation. DO NOT ADD acids or bleach to any liquid wastes containing this product. Avoid breathing vapour. Do not taste or swallow. Use with adequate ventilation. Avoid contact with eyes and skin. Read the MSDS, and follow and handling instructions. Wear appropriate protecte eyewear, clothing and gloves.

 **WARNING ! CHEMICAL HAZARD. Washing Buffer I** Concentrate causes eye, skin and respiratory tract irritation. Avoid brething vapour. Use with adequate ventilation. Avoid contact with eyes and skin. Read the MSDS, and follow the handling instructions; Wear appropriate protective, eyewear, clothing, and gloves.

- Prepare a **3M DTT** solution to perform lysis.

NOTE! You can prepare a 3M DTT stock solution and make aliquots for mid-term storage at -20°C.

- Prepare a **70% ethanol solution**

- Prepare the **Washing Buffers I & II** before first use:
  1. Add 32mL of 96-100% ethanol to the bottle containing 24mL of Washing Buffer I. Homogenize the solution.
  2. Add 39mL of 96-100% ethanol to the bottle containing 17mL of Washing Buffer II. Homogenize the solution.

NOTE! Washing Buffer I & II are delivered concentrated. Before the first use, you have to **add ethanol** in the bottle in the indicated proportion.

3.2. Perform lysis

NOTE! Thaw an aliquot of frozen 3M DTT solution to prepare the lysis.

2.1. From all types of samples except calcified tissues (ex bone powder)

1. Place the sample at the bottom of a microtube.

2. Add **400µL of Crime Lysis Buffer** to the microtube containing the sample.

3. Add **50µL of Proteinase K** to the microtube containing the sample.

4. Add **4.5µL of 3M DTT** to the microtube containing the sample.

NOTE! It is recommended to prepare a fresh premixed lysis solution by combining 400µL of Crime Lysis Buffer, 50µL of Proteinase K and 4.5µL of 3M DTT per test.

Crime Lysis Buffer	400µL	X number of extractions
Proteinase K	50µL	
3M DTT	4.5µL	

In this case, add 454.5µL of premixed lysis solution to the microtube containing the sample.

5. Close the tube, homogenize and place it in a thermomixer, then incubate at +56°C and 1000rpm for 1 hour.

NOTE! For adhesive samples, we recommend an incubation time at +56°C and 1000rpm for 2 hours.

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

6. Perform DNA extraction with 400µL of Lysate.

2.2. From calcified tissues (ex bone powder)

1. Place **100mg of bone powder** at the bottom of a microtube.

2. Add **400µL of Bone Lysis Buffer (#10801)** to the microtube containing the sample.

3. Add **50µL of Proteinase K** to the microtube containing the sample.

4. Add **4.5µL of 3M DTT** to the microtube containing the sample.

NOTE! It is recommended to prepare a fresh premixed lysis solution by combining 400µL of Bone Lysis Buffer, 50µL of Proteinase K and 4.5µL of 3M DTT per test.

Bone Lysis Buffer	400µL	X number of extractions
Proteinase K	50µL	
3M DTT	4.5µL	

In this case, add 454.5µL of premixed lysis solution to the microtube containing the sample.

5. Close the tube, homogenize and place it in a thermomixer, then incubate at +56°C and 1000rpm for 2 hours.

IMPORTANT! The incubation with calcified tissues is 2 hours at +56°C instead 1 hour with the others types of samples.

6. Centrifuge the tube at 10.000rpm (9.500g) for 2 minutes.
7. Perform DNA extraction with the supernatant of the lysate.

3.3. Bind genomic DNA

1. Transfer 400µL of lysate in a new microtube.
Add **250µL of LB Buffer**. Mix well by pipetting.

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2. Add **250µL of isopropanol** and **15µL of Prep-Adembeads**. Mix well by pipetting.

NOTE! It is possible to prepare a premixed solution of Isopropanol / Prep-Adembeads.

Isopropanol	250µL	X number of extractions
Prep-Adembeads	15µL	

Add 265µL of premixed solution.

3. Incubate at room temperature and 1000rpm for 10 minutes.

NOTE! During capture of DNA, it is important to shake in order to improve interactions between DNA and particles.

3.4. Wash bound DNA

After binding DNA to the magnetic particles, wash the magnetic particles to remove impurities and inhibitors. In this protocol, there are **three consecutives washes**.

1. Washing N°1

a. Magnetize the particle suspension at least 5 minutes, and discard carefully the supernatant without disturbing the pellet of magnetic particle.

b. Remove the microtube from the magnet and resuspend the pellet of magnetic particles in **500µL of Washing Buffer I.**

WARNING ! CHEMICAL HAZARD. Washing Buffer I Concentrate causes eye, skin and respiratory tract irritation. Avoid brething vapour. Use with adequate ventilation. Avoid contact with eyes and skin. Read the MSDS, and follow the handling instructions; Wear appropriate protective, eyewear, clothing, and gloves.

2. Washing N°2

a. Magnetize the particle suspension at least 5 minutes, and discard carefully the supernatant without disturbing the pellet of magnetic particle.

b. Remove the microtube from the magnet and resuspend the pellet of magnetic particles in **500µL of Washing Buffer II.**

3. Washing N°3

a. Magnetize the particle suspension at least 5 minutes, and discard carefully the supernatant without disturbing the pellet of magnetic particles.

b. Remove the microtube from the magnet and resuspend the pellet of magnetic particles in **500µL of 70% ethanol**.

3.5. Drying

1. Magnetize the particle suspension at least 5 minutes.

2. Eliminate carefully the supernatant without disturbing the pellet of magnetic particles.

3. Let the pellet of magnetic particles dry for 5 minutes.

NOTE! Magnetization and Drying times are given as an indication.

4. Remove the liquid remaining in the bottom of the tube by pipetting

3.6. Elute DNA

1. Remove the microtube from the magnet and resuspend thoroughly the pellet of magnetic particles in **60µL of Elution Buffer**.

IMPORTANT! Do not use water instead of Elution Buffer.

2. Incubate the microtube at 75°C and 1000rpm for 10 minutes.

3. Place the microtube on the magnet for at least 5 minutes.

4. Collect the supernatant containing pure DNA and transfer it to another microtube.

NOTE! Store or analyze the purified DNA accordingly. If DNA is not analyzed immediately, store it at 4°C for up to 24 hours. For longer period, consult laboratory guidelines. Freezing samples at -20°C has been shown to preserve DNA for longer periods of time.

Troubleshooting

Observations	Possible cause	SUGGESTION
Magnetic particles settled in the bottle.	During shipping, magnetic particles settled.	Thoroughly flick / vortex the bottle. Prep-Adembeads are stored between+2-8°C, before using incubate them at room temperature.
Supernatants contain magnetic particles.	The magnetic stand used is not adapted to the magnetic particles. Incorrect position for microtubes in the sample holder	Keep the tube containing magnetic particles in the magnet for at least 5 minutes
DNA eluate contains magnetic particles	Aggressive pipetting can disturb magnetic pellet	Keep the tube containing magnetic particles in the magnet for at least 5 minutes then pipette out carefully the supernatant
Nor or low yield of DNA	Biological sample contains no or low amount of DNA	Review protocol steps and reagents additions
		Extract DNA from a different cutting from sample
	Insufficient amount of magnetic particles added	Review protocol steps and reagents additions

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

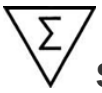
Reference Number

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.

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
06211	Crime Prep Adem-Kit	10X96
06212	Crime Prep Adem-Kit AutoMag Solution (prefilled reagents plates)	48 (4X12)
06140	Smart D-N-Adem-Kit for Profiling	1 x 100
10801	Bone Lysis Buffer	48

- **Instruments**

CAT NO.	PRODUCT	PACKAGE SIZE
20105	Adem-Mag MODULO Classic	Each
20106	Adem-Mag 96	Each
20108	Adem-Mag MODULO Brick	Each