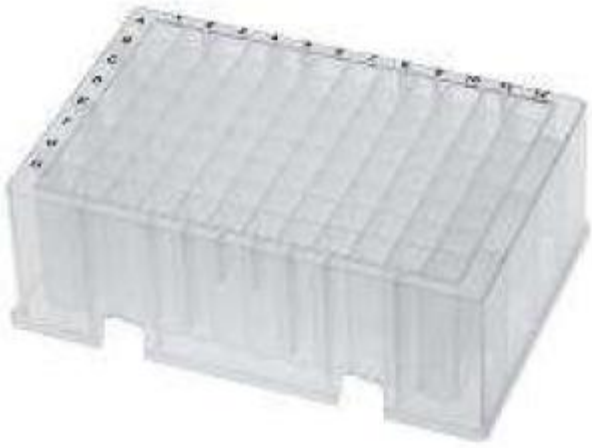
NA Extraction Kit on High Throughput Automate
Quick Overview

High Throughput Extraction Crime Prep Adem-Kit 1 x 96 (#06213)

Magnetic Handling Systems & Liquid Handling Systems



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Crime Prep Adem-Kit (#06213)
On
High Throughput Systems
Quick Overview

The Crime Prep Adem-Kit reagents

INTENTED USE

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.

KIT CONTENT

Each Crime Prep Adem-Kit contains sufficient reagent to perform 96 samples using the following standard protocol. The contents of the kit are described in the table below.

Item	Crime Prep Adem-Kit
Package size	**96 samples**
Crime Lysis Buffer	**40 mL**
Proteinase K (10mg/ml)	**5 mL**
Prep-Adembeads (x4)	**410 µL (x4)**
LB Buffer	**35 mL**
Isopropanol	**35 mL**
Washing Buffer I	**56 mL**
Washing Buffer II	**56 mL**
Ethanol 70%	**56 mL**
Elution Buffer	**8 mL**

Storage conditions: The kits are shipped and stored at room temperature (+4-25°C)

STABILITY AND STORAGE CONDITION

The kits are sent 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 condition
All reagents can be stored between +4°C and +25°C except for the DTT and the Proteinase K which has to be absolutely stored at +2-8°C. If you store the whole kit at +4°C, before using, 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.

CAUTION AND WARNING

WARNING ! CHEMICAL HAZARD. LB Buffer and Washing Buffer I in contact with acids or bleach liberate 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 protect eyewear, clothing and gloves..

MATERIALS AND REAGENTS NEEDED

Handling chemicals requires wearing a labcoat, gloves and goggles. To avoid contaminations wear a mask.

Items needed but available separately:

- Forensic Filters (product #12102)
- DeepWell Plate (product #21102)
- 96-tip Comb (product #21105)
- 1M DTT (product #43816)

Materials needed but not provided:

- Sterile pipettes and tips (volumes ranging from 10 µL to 1 mL)
- Vortex
- Thermomixer for microtubes (56°C)
- High Speed Centrifuge for microtubes

Magnetic Handling Systems KingFisher Flex

PRECAUTIONS

I) Before Using

- Do not operate KingFisher Flex without qualified operation training.
- Read user's manual carefully before operation.

II) Handling Requirement

- Do not use a kit after its expiration date.
- Do not touch the reagents with bare hands. Keep away from your skin, eyes, or mucous membranes. If contact does occur, wash the affected area immediately with large amounts of water. If you spill the reagents, dilute the spill with water before wiping it up.
- Do not allow reagents to mix with sodium hypochlorite solution or strong acids. This mixture can produce a highly toxic gas.

III) Laboratory Procedures

- Handle all samples and the resulting waste as if potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample material varies, the operator has to optimize pathogen inactivation by the Binding Buffer or take appropriate measures according to local safety regulations. Ademtech does not warrant that samples treated with Binding Buffer are completely inactivated and noninfectious. After sample processing is completed, remove and autoclave all disposable plastics.
- Do not eat, drink or smoke in the laboratory working area.
- Wear protective disposable gloves, laboratory coats and goggles when handling samples and kit reagents.
- Do not use sharp or pointed objects when working with the reagent cartridges, this is to prevent damage of the sealing foil and loss of reagent.
- Do not contaminate the reagents with bacteria, virus, or ribonuclease. Use disposable pipettes and RNase-free pipette tips only to remove aliquots from reagent bottles.
- Use the general precautions described in the literature.
- Wash hands thoroughly after handling samples and test reagents.

IV) Waste Handling

Discard unused reagents and waste according to country, federal, state and local regulations.

PLASTIC CONSUMABLE NEEDED (can be supplied by Ademtech)

Name	Quantity	Material
Deepwell plates for 96 samples (#21102)	**6**	
96 Tip-comb for DW96 (#21105)	**1**	

PROTOCOL

Read the complete protocol carefully before starting.

It is strongly recommended to decontaminate the workbench and the equipment (UV / disinfectant) before using the kit.

Good laboratory practices are needed.

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.

- **<u>Lysis of the sample</u> :**

  1. Transfer the sample into a forensic filter (#12102)
  2. Add 400µl of Crime Lysis Buffer in the microtube containing the sample
  3. Add 50 µl of Proteinase K
  4. Add 15µL of 1M DTT

NOTE! It is recommended to prepare a fresh premixed lysis solution by combining 400µL of Crime Lysis Buffer, 50µL of Proteinase K and 15 µL of 1M DTT per test. In this case, add 465 µL 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. Centrifuge the column for 2 min at 11.000 rpm, eliminate the filter.

- **<u>DNA Extraction on KingFisher Flex</u> :**

1. Prepare 6 DeepWell plate by the volume indicated below in each well

DW plate	Reagents	Volume / well
Elution Plate	**Elution Buffer**	**60 µL**
Ethanol 70%	**Ethanol 70%**	**500 µL**
Washing Buffer II	**WBII**	**500 µL**
Washing Buffer I	**WBI**	**500 µL**
Lysis Binding	**LB Buffer**	**250 µL**
	Isopropanol	**250 µL**
	Magnetic Beads	**15 µL**
Tip Plate	**place a 96 Tip comb in a Deep Well plate**	

2. Add 465 µL of lysate into the Lysis Binding Plate
3. Start the KingFisher Crime Prep Program and follow the instruction by adding each plate in the corresponding rack
4. Place the DW plate containing the lysate into the automate and start the automated extraction
5. At the end of the run, retrieve the Elution Plate containing the eluate and store it using proper conservation condition

NOTE! If the DNA is not analysed immediately, it can be stored at 4°C until 24h. For longer storage, consult the laboratory instructions. It has been shown that freezing sample eluates at -20°C allows conservation over a longer period.

Liquid Handling Systems

MATERIALS NEEDED on Liquid Handling System (not provided)

Name	Quantity	Material
96w ring magnet	1	
Heater Shaker (3mm) and DeepWell Adaptator	1	
Rack 16 tubes	13	
Container (100ml)	4	
Container (25 ml)	1	
Filter Tips (1000 µL)	5	
Filter Tips (200 µL)	2	
Filter Tips (50 µL)	1	

PROTOCOL

Read the complete protocol carefully before starting.

It is strongly recommended to decontaminate the workbench and the equipment (UV / disinfectant) before using the kit.

Good laboratory practices are needed.

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.

- **Lysis of the sample :**

1. Transfer the sample into a forensic filter (#12102)
2. Add 400µl of Crime Lysis Buffer in the microtube containing the sample
3. Add 50 µl of Proteinase K
4. Add 15µL of 1M DTT

NOTE! It is recommended to prepare a fresh premixed lysis solution by combining 400µL of Crime Lysis Buffer, 50µL of Proteinase K and 15 µL of 1M DTT per test. In this case, add 465 µL 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. Centrifuge the column for 2 min at 11.000 rpm, eliminate the filter.

- **<u>Extraction on the Liquid Handling System</u> :**

1 Initialization:

- Manual preparation of mix LB/Isopropanol (v50/50)
- Installation of material and reagents (Buffers, Tubes, Racks)
- Transfer of the Deepwell 96 plate (DW96) on the heater/shaker (HSS)
- Homogenization of magnetic particules “Prep-Adembeads” (Mix 200µL 5 times)
- Distribution of Prep-Adembeads into lysates tubes (15µL)
- Distribution of LB/Isopropanol into DW96 (500µL)

2 Capture:

- HSS : Distribution of Lysates/Prep-Adembeads into DW96 (480µL)
- HSS : 600 sec, 1200 rpm, RT (3x200 sec / clockwise (CW) /counterclockwise (CCW))
- Transfer DW96 to Magnet
- Magnet : 420 sec
- Magnet : Elimination of supernatant (Aspiration 530µL / Dispense 520µL / 2 times)
- Transfert DW96 to HSS

3 Washing 1:

- HSS : Distribution of WBI into DW96 (500µL)
- HSS : 240 sec, 1200 rpm, RT (8x30 sec, CW/CCW)
- Transfer DW96 to Magnet
- Magnet : 150 sec
- Magnet : Elimination of supernatant (Aspiration 280µL / Dispense 270µL / x2)
- Transfert DW96 to HSS

4 Washing 2:

- HSS : Distribution of WBII into DW96 (500µL)
- HSS : 240 sec, 1200 rpm, RT (8x30 sec, CW/CCW)

- Transfer DW96 to Magnet
- Magnet : 150 sec
- Magnet : Elimination of supernatant (Aspiration 280µL / Dispense 270µL / x2)
- Transfert DW96 to HSS

5 Washing 3 (Ethanol 70%):

- HSS : Distribution of Ethanol 70% into DW96 (500µL)
- HSS : 240 sec, 1200 rpm, RT (8x30 sec, CW/CCW)
- Transfer DW96 to Magnet
- Magnet : 150 sec
- Magnet : Elimination of supernatant (Aspiration 280µL / Dispense 270µL / x2)
- Transfert DW96 to HSS

6 Drying:

- *Set HSS temperature to 75°C*
- Magnet : 300 sec
- Magnet : Elimination of supernatant (90µL)
- Magnet : 300 sec
- Transfert DW96 to HSS

7 Elution:

- HSS : Distribution of elution buffer (60µL)
- HSS : 600 sec, 1200 rpm, 75°C
- Transfer DW96 to Magnet
- Magnet : 300 sec
- Magnet : Transfer of eluates into collection tubes (60µL)

LIQUID CLASSES DEFINITION

Liquid Class	Range	Movement	Speed	Position	Offset	Liquid Detection	Note
Beads	100-1000 µL	Aspirate	40 µL/s	Liquid Level	3 mm	Yes	
		Dispense	110 µL/s	Liquid Level	3 mm	Yes	
	25-100 µL	Aspirate	50 µL/s	Liquid Level	3 mm	Yes	On error go to z-max
		Dispense	225 µL/s	z-max	-5 mm	No	
Main	100-1000 µL	Aspirate	50 µL/s	z-max	0 mm	No	
		Dispense	300 µL/s	z-dispense	0 mm	No	
	25-100 µL	Aspirate	40 µL/s	z-max	-0,5 mm	No	
		Dispense	500 µL/s	z-dispense	0 mm	No	
Elution	50-100 µL	Aspirate	70 µL/s	Liquid Level	2 mm	Yes	On error user prompt
		Dispense	225 µL/s	z-dispense	0 mm	No	

TROUBLESHOOTING

Comments	Possible causes and suggestions	
An error message appears on the instrument	Refer to the manual of the KingFisher instrument	
No or little DNA recovered	The biological sample contained little or no DNA initially.	Re-read the protocol carefully.
	Adapt the lysis	Cf NOTE p.7
DNA eluate contain magnetic particles	Bad magnetization after elution	Eliminate magnetic particles (magnetization or centrifugation). Recover the eluate.

SYMBOLS

Product code

Lot number of the product
This symbol is accompanied by the manufacturer's batch number


Manufacturer
This symbol is accompanied by the name and address of the manufacturer of the product


Expiration date
This symbol is accompanied by the expiry date of the product

Sufficient content for (n) tests
The kit is intended for the number of extraction indicated in the symbol.


Storage temperature
The minimum and maximum temperatures are indicated at the horizontal bar.

LIMITATIONS

- All reagents are intended for RUO.
- Strict comliance to the instructions in the operating instructions is necessary to ensure optimum results.

ORDERING INFORMATIONS

- **Ademtech Kits**

CAT NO.	PRODUCT	FORMAT
06213	Crime Prep Adem-Kit	96 tests
12102	Forensic Filters	250 pc
21102	DeepWell Plate	50 pc
21105	96 Tip-comb	100 pc
43816	1M DTT	10 mL