

AutoMag Solution

Version 1.2

REF 06212

Instruction manual for automation protocol

Prefilled reagents for the AutoMag Instrument., the kit is used for for optimal DNA extraction from forensic casework samples

Kit for 48 reactions

ADEMTECH SA Bioparc BioGalien 27, allée Charles Darwin 33600 PESSAC

France

Tel: +33557020201 Fax +3355702020

Visit our Web site: www.ademtech.com

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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.

2. Product Component and storage conditions

<u>Kit Content:</u> Each AutoMag Crime Prep Adem-Kit contains sufficient reagents to perform 48 extractions. The content of the kit are described in Table

| Item | Description | Storage condition | Included with Crime Prep Adem- kit for AutoMag Solution (#06212) |
|----------------------------|--|----------------------|---|
| Crime Prep Lysis Buffer | One bottle, 20ml | +2-8°C | • |
| Proteinase K | One bottle, 2.5ml, (concentration 10mg/ml) | +2-8°C | • |
| DNA extraction plate | 4 prefilled plates per kit | + 20°C | • |
| AutoMag Tip Combs | 4 X12-tip comb for 96 Deepwell plate per kit | +20°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 products.

Before using the Crime Lysis Buffer, it is recommended to take out in advance and check if there are any precipitates. If the Buffer present precipitates place them at room temperature and eventually put them at +37°C.

WARNING! CHEMICAL HAZARD. DNA Extraction Plate may contain the following: guanidine thiocyanate, isopropanol, and ethanol. Refer to MSDS, and follow the handling instructions; and disposal.

WARNING! CHEMICAL HAZARD. DNA Extraction Plate contains LB Buffer (row C) which 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 protect eyewear, clothing and gloves.

WARNING! CHEMICAL HAZARD. DNA Extraction Plate contains Washing Buffer (row D) which concentrate causes eye, skin and respiratory tract irritation. Avoid breathing 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.

3. Required reagents and materials

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 required but not provided:

• 3M Dithiothreitol (DTT)

Materials required but not provided:

- 56°C heat block, thermomixer or water bath
- Microtubes
- AutoMag Instrument (Cat.# 21101) (includes the Crime Prep Adem-Kit script)

Acessory products:

The following products are available for purchase separately from Ademtech

| CAT NO. | PRODUCT | PACKAGE SIZE |
|---------|-----------------------------------|------------------|
| 21101 | AutoMag Instrument | Each |
| 21102 | Microtiter Deepwell 96 plate | 1 pack of 50 pcs |
| 21103 | 12-tip comb for 96 Deepwell plate | 1 pack of 50 pcs |
| 10801 | Bone Lysis Buffer | 48 extractions |

Crime Prep Adem-Kit Protocol

The standard protocol is appropriate for all kind of sample types, such as samples on solid support 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.

1. Reagents preparation

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.

2. Prepare sample lysates

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.
- 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 400 μ L of Crime Lysis Buffer, 50 μ L of Proteinase K and 4.5 μ L of 3M DTT per test.

Crime Lysis Buffer $400\mu L$ Proteinase K $50\mu L$ X number of extractions 3M DTT $4.5\mu L$

In this case, add $454.5\mu L$ 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.
- 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 400 μ L of Bone Lysis Buffer, 50 μ L of Proteinase K and 4.5 μ L of 3M DTT per test.

Bone Lysis Buffer $400\mu L$ Proteinase K $50\mu L$ X number of extractions 3M DTT $4.5\mu L$

In this case, add $454.5\mu L$ 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 Lysate on AutoMag Instrument.

3. Set up and run automated DNA extraction

Required materials for extraction:

- AutoMag Instrument (#21101)
 - AutoMag Instrument is a low-to-medium-throughput purification system with a magnetic particle processor for DNA purification kits. With the AutoMag Instrument customers can process up to 12 samples per run with a working volume up to 1 ml. In addition, it is possible to run two purification methods sequentially without interruption, raising the throughput up to 24 samples
- Crime Prep Adem-kit Protocol script "Crime Prep v1.3"

- From the appropriate kit (Crime Prep Adem-Kit AutoMag Solution #06212)
 - The "DNA Extraction Plate"
 The plates are designed to fit onto the AutoMag rack in only one orientation. Plate are sealed and pre-filled with the Crime Prep Adem-Kit reagents required for the protocol.
 Row B and G are empty
 - AutoMag Tips combs



AutoMag Instrument (Cat.# 21101)

Set up the AutoMag instrument for DNA extraction

- 1. Prepare the lysate according to the instructions on page 7.
- 2. Inspect the DNA Extraction plate.

NOTE!

One sealed and prefilled plate is required per 12 samples.

Before take-off the adhesive film from the top of the pre-filled reagent plate, briefly centrifuge in order to move all the reagents to the bottom.

Gently remove the film of the pre-filled reagents plate.

Table 1 Summary of DNA Extraction Plate content

| Row | Row name | Content |
|-----|--------------------|------------------|
| Α | Elution | Elution Buffer |
| В | Tip | empty |
| 0 | 0 1 | LBB buffer |
| С | Capture | Isopropanol |
| D | Wash 1 | Washing Buffer 1 |
| E | Wash 2 | Washing Buffer 2 |
| F | Wash 3 | 70% ethanol |
| G | Empty | Empty |
| Н | Magnetic particles | Prep-Adembeads |

NOTE!

Row B is reserved for the tip comb

Row G is left empty

Transfer 400 μ l of Lysate in each well of the Row C (less than 400 μ l can be recovered depending of the sample, in this case use all the volume of samples).

3. Place a 12-tip comb into row B of the prefilled reagents plate.



12-tip comb for 96 Deewell plate (Cat.# 21103)

NOTE!

Only one tip comb can be placed onto a DNA extraction plate

4. Load the plate in the AutoMag Instrument and start the "Crime Prep v1.3".

Switch ON the AutoMag Instrument and make sure that you are using the 12-pin magnet head and heating block.

Select the protocol by using the arrows keys and press **START**Load the pre- filled reagents plate as indicated on the AutoMag

Instrument display and press OK.

NOTE! Place the A1 well of the sealed plate ensuring that it is in the upper right corner. The first A1 row is consequently always in the inner circle

5. After the run is completed, remove the DNA Extraction Plate and store the purified DNA.

When the protocol is completed, remove the DNA Extraction Plate according to the instructions on the AutoMag Instrument display and turn off the instrument.

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 | Comments and suggestions | |
|---|--|--|
| Error message in instrument display | Refer to the user manual supplied with your AutoMag Instrument. | |
| The tip comb holder lifting mechanism is out of position | Switch the instrument OFF and ON, and try again. If the error appears during initialization or is otherwise repeated, contact service. | |
| The turntable rotating mechanism is out of position | Switch the instrument OFF and ON, and try again. If the error appears during initialization or is otherwise repeated, contact service. | |
| The magnet head holder lifting mechanism is out of position | Switch the instrument OFF and ON, and try again. If the error appears during initialization or is otherwise repeated, contact service. | |
| The plastic tip comb is not attached to the holder | Check if the tips are presents. If it looks all right, turn ON and OFF, and run the check protocol. | |
| 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 the sample |

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





The symbol is accompanied by the manufacturer's batch code



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



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.



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



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 |
|---------|--|--------------|
| 06210 | Crime Prep Adem-Kit | 1 x 96 |
| 06212 | Crime Prep Adem-Kit AutoMag Solution (prefilled 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 |