Smart D-N-Adem-Kit

for Profiling

AutoMag Solution

Instruction manual for automation protocol from

FTA cards (cat #06140)

Swabs (cat #06142)

ADEMTECH SA
Bioparc BioGalien
27, allée Charles Darwin
33600 PESSAC

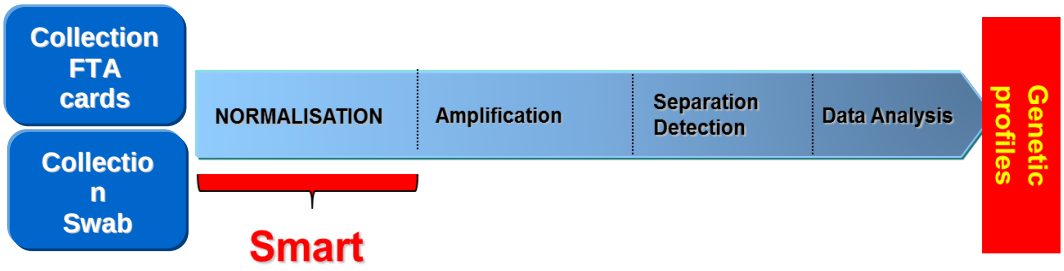
France

Tel: +33557020201
Fax +33557020206

Visit our Web site: www.ademtech.com

Introduction

DNA quantities present on FTA cards or swab vary from sample to sample, the collecting devices used, the collection methods applied, the swab-to-FTA® transfer protocol and also from laboratory to laboratory. Blood and buccal samples often contain substances that can inhibit DNA amplification. Ademtech has developed the Smart D-N-Adem-Kit profiling for delivering a consistent amount of pure DNA to considerably enhance quality profile and efficiency of forensic laboratories. The DNA is ready to use for STR amplification without any added quantification steps.



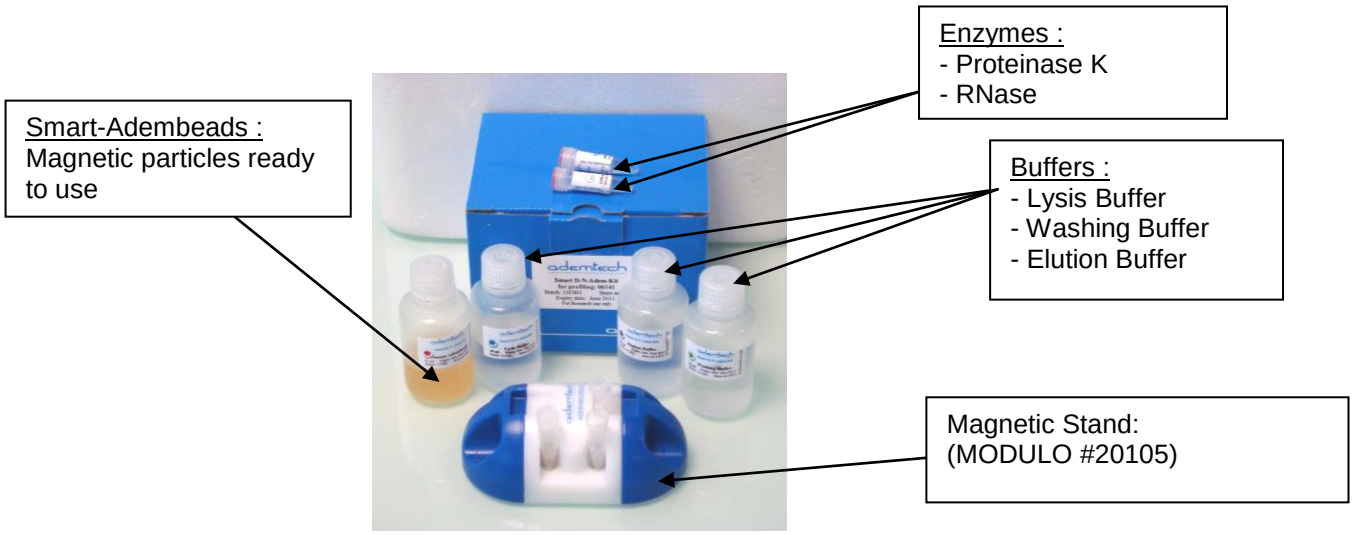
1. Smart D-N-Adem-Kit for profiling

1.1. Smart-Adembeads Description

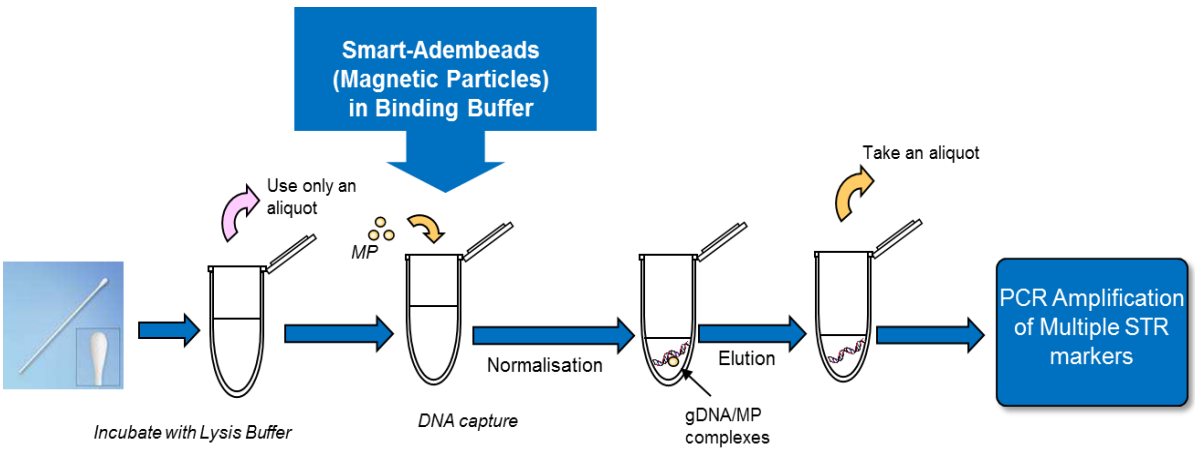
Smart-Adembeads are uniform, monosized beads of 300nm with a large and well defined specific area that ensure optimal reproducibility. Their capacity and performance lead to the capture of a consistent amount of DNA. The beads are composed of a magnetic core encapsulated by a highly cross-linked polymer shell. The high iron oxide content (70%) increases magnetic strength of the beads and ensures rapid magnetic mobility and efficient isolation of nucleic acids. The nanosized beads feature a very low sedimentation rate ideal for fast reaction kinetics, making them particularly suitable for automated assays. Alternative particles from other suppliers often present a random size range distribution, a porous surface associated with an irregular binding capacity; these compromise the reproducibility of your assays.

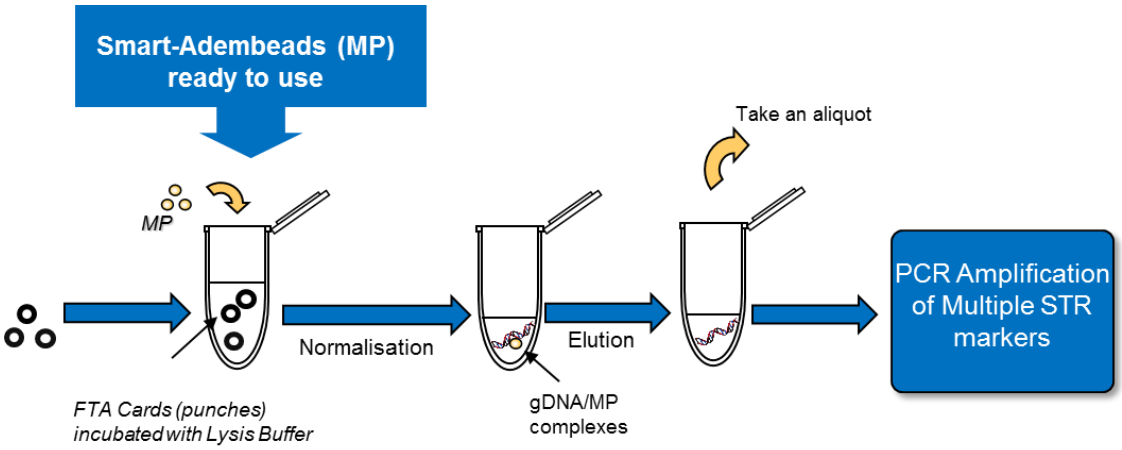
1.2. Smart D-N-Adem-Kit Description

The Smart D-N-Adem-Kit contains Smart-Adembeads and specific buffers optimized for capture and normalisation of DNA. Smart-Adembeads offer an innovative surface for gDNA capture and compatible with a direct amplification. The Smart D-N-Adem-Kit procedure allows cleaning gDNA and avoids the use of phenol, ethanol, chloroform and ionic chaotropes that could inhibit PCR.



2. DNA normalisation procedure overview





3. Kit contents and storage conditions

NOTE ! Smart D-N-Adem-Kit avoids the use of harmful organic solvents such as phenol, ethanol, isopropanol or guanidine thiocyanate that can react with acids and bases and generate toxic gas, and eliminates multiple centrifugation steps used in some purification procedures.

Kit contents: Each Smart D-N-Adem-Kit from FTA cards contains sufficient materials to perform 100 or 400 normalisations using the following standard protocol.

Table 1: *Materials provided within Smart D-N-Adem-Kit for Profiling (# 06140)*

Smart D-N-Adem Kit (#06140)			
	Amount	Reagents	Storage conditions
R1	50µl	RNase A	+ 4°C
R2	250µl	Proteinase K	+ 4°C
R3	6ml	Lysis Buffer	+ 4°C
R4	10ml	Smart-Adembeads	+ 4°C
R5	10ml	Washing Buffer	+ 4°C
R6	10ml	Elution Buffer	+ 4°C
contains sufficient reagents to perform **100 normalisations**			

Storage conditions: The kits are shipped at room temperature and stored at 4°C to 8°C upon reception.

Kit contents: Each Smart D-N-Adem-Kit from Swab contains sufficient materials to perform 100 normalisations using the following standard protocol.

Table 3: *Materials provided within Smart D-N-Adem-Kit for Profiling (# 06142)*

Smart D-N-Adem-Kit (#06140)			
	Amount	Reagents	Storage conditions
R1	50µl	RNase A	+ 4°C
R2	250µl	Proteinase K	+ 4°C
R3	50ml	Lysis Buffer	+ 4°C
R4	50ml	Smart-Adembeads	+ 4°C
R5	10ml	Washing Buffer	+ 4°C
R6	10ml	Elution Buffer	+ 4°C
contains sufficient reagents to perform **100 normalisations**			

Storage conditions: The kits are shipped at room temperature and stored at 4°C to 8°C upon reception.

NOTE ! 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.

IMPORTANT ! Do not freeze the magnetic particles.

Smart D-N-Adem-Kit Protocol

1. Smart-Adembeads Guidelines

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

- The Smart-Adembeads are ready to use (there is no need to add Binding Buffer). Colour of the solution is due to the presence of the magnetic particles.

2. Smart D-N-Adem-Kit Protocol

Before starting gDNA extraction procedure, all buffers shall be at room temperature (20-25°C) for optimal performances.

2.1. Prepare samples from FTA cards

The standard protocol is appropriate for blood or buccals cells on FTA cards.

Sample type	Example sample input
Blood on FTA	5 to 10 mm^2 cutting or 1-2 punches
Buccals cells on FTA	10 to 30 mm^2 cutting 3 punches (3,2mm diameter) 6 punches (1.2mm diameter)

1. Punch out sample and place in a new microplate.
2. Perform steps 2*a* through 2*c* twice.
    a. Add 150µl of Nuclease Free Water.
    b. Incubate 5 minutes at 800 rpm at room temperature.
    c. Discard the supernatant.

IMPORTANT! Washing punches for more than 2 times may reduce DNA yield. Incubation time can be varied from 2 min to 1 hour.

2.2. Perform lysis from FTA cards

Before starting gDNA extraction procedure, all buffers shall be at room temperature (20-25°C) for optimal performances.

Preparation of Lysis solution : Prepare the Lysis solution by combining Lysis Buffer, Proteinase K and RNase in the proportions as indicated below. Mix by pipetting or vortex the tube.

Lysis Buffer	60µl
Proteinase K solution	2,5µl
RNase A solution	0,5µl
Total volume:	**63µl**

Prepare 63µl of Lysis solution for each FTA Cards.

IMPORTANT! RNase must be added in the last to avoid its early degradation by Proteinase K.

Perform lysis: After preparing the solution, perform extraction.

1. Set the thermal shaker temperature to 56°C.
2. Add **60µl of freshly-prepared Lysis solution** to the microplate or microtube containing the washed punches.
3. Place the tube or the microplate in a thermal shaker, then incubate at 56°C and 800 rpm for 30 minutes.

   IMPORTANT! Temperature can be varied between 50°C and 60°C. You can use a heat block instead of a thermal shaker, however the DNA yield may be lower. For effective recovery, make sure that the sample is immerged by the Lysis Solution during mixing.

Note! During incubations, we recommend to use a lid to close the microplate indeed a plastic film to avoid droplet cross-contamination. If you use plastic film, don't forget to centrifuge microplate before removing the film.

4. Use only **50µl of lysat** to perform DNA extraction.

2.3. Prepare samples from Swab

The standard protocol is appropriate for buccals cells on swabs (all types of swabs dedicated to forensic including Omniswab ™, Cotton swab).

1. Place the extremity of the dry swab in a 2mL microtube.
2. Break the swab shaft in order to close the tube or push the head of the swab in the bottom of the microtube.

2.4. Perform lysis from Swab

Preparation of Lysis solution: Prepare the Lysis solution by combining Lysis Buffer, Proteinase K and RNase in the proportions as indicated below. Mix by pipetting or vortex the tube.

Lysis Buffer	500µl
Proteinase K solution	2,5µl
RNase A solution	0,5µl
Total volume:	**503µl**

Prepare 503µl of Lysis solution for each swab.

IMPORTANT! RNase must be added in the last to avoid its early degradation by Proteinase K.

Perform lysis:

1. Add **500µl of freshly-prepared Lysis solution** to the microtube containing the head of the swab.
2. Mix vigorously for 5 seconds.
3. Place the tube in a thermal shaker, then incubate at 56°C at 800 rpm for 30 minutes.

IMPORTANT! Temperature can be varied between 50°C and 60°C. You can use a heat block instead of a thermal shaker, however the DNA yield may be lower. For

effective recovery, make sure that the sample is immerged by the Lysis Solution during mixing.

4. After the incubation, mix vigorously for 5 seconds.
5. Use only **50µl of lysat** to perform DNA extraction.

Note! During incubations, we recommend to use a lid to close the microplate indeed a plastic film to avoid droplet cross-contamination. If you use plastic film, don't forget to centrifuge microplate before removing the film.

2.5. Instruction for AutoMag Instrument with 12-pin magnet head and 96 deep well plates for DNA extraction from forensic samples

These instructions are for the DNA extraction from 50µl of lysate using the Smart D-N-Adem-Kit from FTA cards (Cat.# 06140) or from 50µl of lysate using the Smart D-N-Adem-Kit from Swab (Cat.# 06142), and the AutoMag Instrument (Cat.# 21101) with a 12−pin magnet head and 96 deep well plates.

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.

AutoMag Instrument (Cat.# 21106)

Instructions:

1. **Prepare the lysate according to the instructions.**

2. **Take one empty Microtiter Deep well 96 plate and one Elution strip.**

Microtiter Deep well 96 plate
(Cat.# 21102)

Elution strip
(Cat.# 21104)

3. Prepare the plate

Add the following reagents to the rows. ***Note that row B is reserved for the tip comb and should be left empty****. Note that row D, E, F, G and H are left empty.*

Plate name and type	Row	Row name	Content	Reagent/Sample volume per well
Microtiter Deep well 96 plate	A	Sample	Lysate Smart Adembeads	50µL 100µL
	B	Tip	12-tip comb	Empty
	C	Wash	Washing Buffer	100µL
	D	Empty	Empty	Empty
	E	Empty	Empty	Empty
	F	Empty	Empty	Empty
	G	Empty	Empty	Empty
	H	Empty	Empty	Empty

NOTE! Fill up the **Row A** with **100µL Smart Adembeads solution** and **50µL of Lysate**.

4. Fill the Elution strip as follows.

Make sure that the elution strip is placed in the correct direction into the elution block. Ensure that the perforated end is facing towards the user and the Elution Buffer is pipetted into the correct wells.

Elution strip	Content	Reagent volume per well
Elution strip	Elution Buffer	- 80µL for FTA cards procedure - 100µL for Swab procedure

5. Place a 12-tip comb for 96 Deepwell plate into row B of the plate.

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

6. **Start the "Smart for profiling v1.0" protocol with the AutoMag Instrument and load the plate and elution strip.**

   *Switch ON the AutoMag Instrument and make sure that you are using the 12-pin magnet head and heating block. Start the "Smart for profiling v1.0" protocol. Insert the plate and elution strip into the instrument as indicated on the AutoMag Instrument display and press **OK**. Make sure that the elution strip is placed in the correct direction into the elution block. Ensure that the perforated end is facing towards the user.*

7. **After the run is completed, remove the plate and store the purified DNA.**
   When the protocol is completed, remove the plate and elution strip according to the instructions on the AutoMag Instrument display and turn off the instrument.

NOTE! During the elution step, the heating leads to 10-25% of liquid evaporation. This has the affect of concentrating the extracts.

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.

Summary of plate and elution strip content

Plate name and type	Row	Row name	Content	Reagent/Sample volume per well
Microtiter Deep well 96 plate	A	Sample	Lysate	50µL
			Smart Adembeads	100µL
	B	Tip	12-tip comb	Empty
	C	Wash	Washing Buffer	100µL
	D	Empty	Empty	Empty
	E	Empty	Empty	Empty
	F	Empty	Empty	Empty
	G	Empty	Empty	Empty
	H	Empty	Empty	Empty
Elution strip		Elution	Elution Buffer	- 80µL for FTA cards procedure - 100µL for Swab procedure

DNA analysis and expected results / FTA cards

For quantifying extracted DNA	■ We recommend using quantification PCR kit which provides a rapid, sensitive, and accurate method for dsDNA quantification instead of UV absorbance. ■ For quantification take an aliquot of 2µl for a 25µl PCR reaction.
For STR analysis	■ For STR amplification, we recommended using between 3-7µl of DNA eluate.

DNA analysis and expected results / Swab

For quantifying extracted DNA	■ We recommend using quantification PCR kit which provides a rapid, sensitive, and accurate method for dsDNA quantification instead of UV absorbance. ■ For quantification take an aliquot of 1µl or 2µl for a 25µl PCR reaction.
For STR analysis	■ For STR amplification, we recommended using between 1-2µl of DNA eluate.

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.