Smart D-N-Adem-Kit

for Profiling

(cat #06140)

Instruction manual for
gDNA normalisation from FTA™ cards

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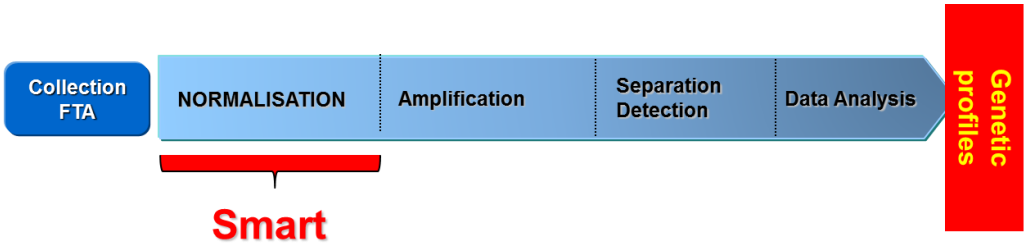
Introduction

DNA quantities present on FTA™ cards 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.



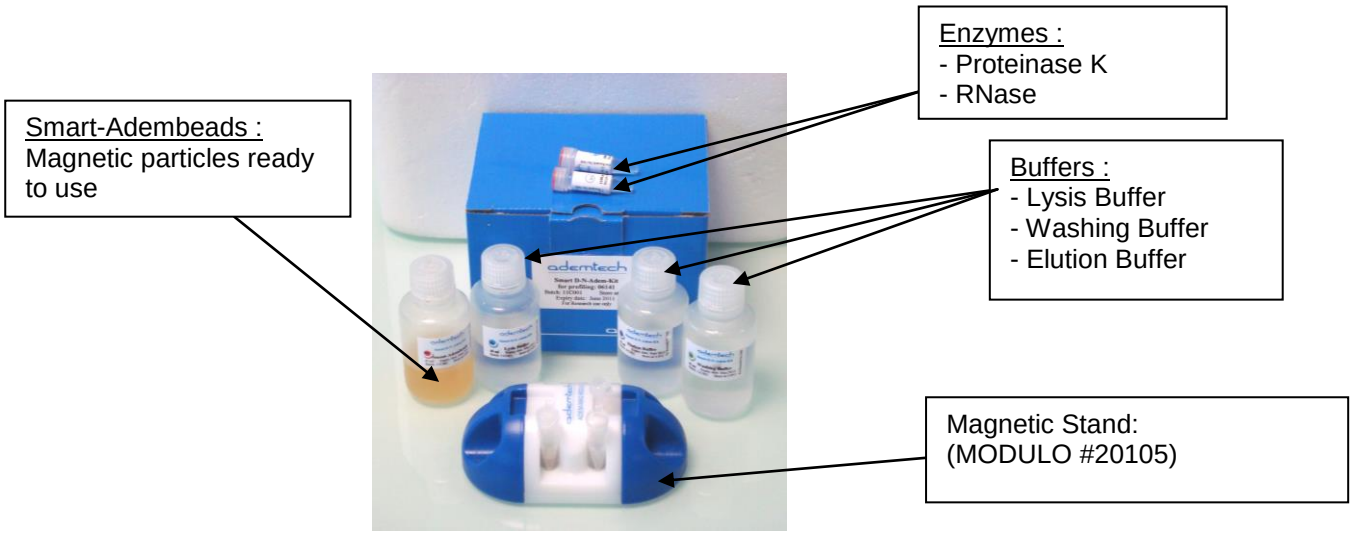
1. Smart D-N-Adem- Kit for profiling

1.1. Smart-Adembeads Description

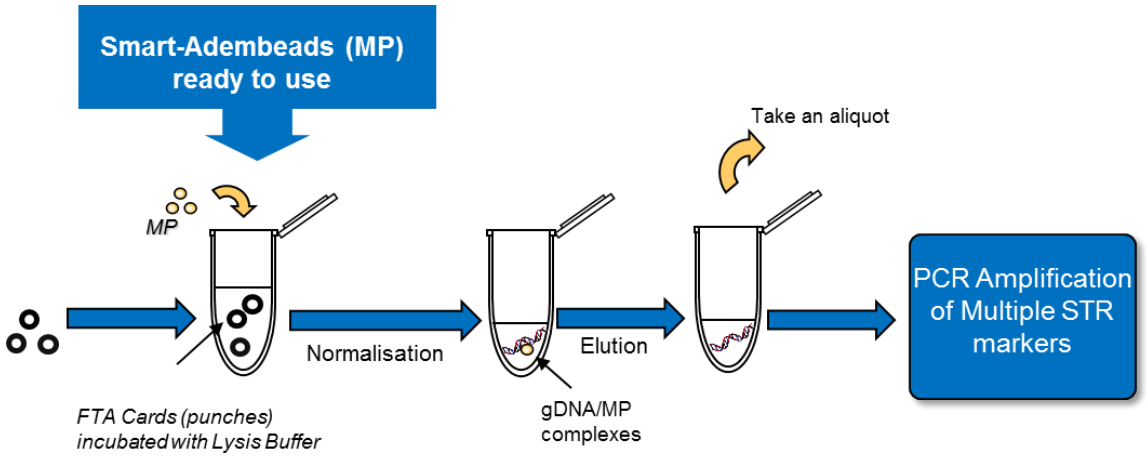
Smart-Adembeads are uniform, monosized beads of 300nm with a large and well defined specific area that ensure optimal reproducibility. Their capacity and performance lead to the capture of a consistent amount of DNA. The beads are composed of a magnetic core encapsulated by a highly cross-linked polymer 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 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**			

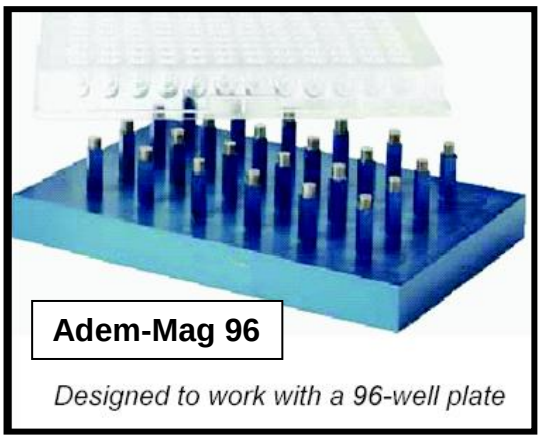
Storage conditions: The kits are shipped at room temperature and stored at 4°C to 8°C upon reception.

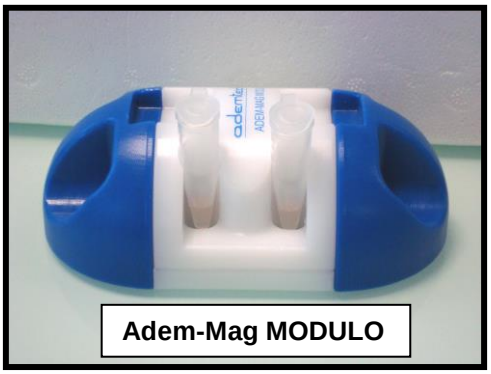
NOTE ! Properly stored Kits are guaranteed until the expiry date. Note that shipping is realized at room temperature and will not affect stability. All components of the kit have been prepared under nucleases free conditions and have been thoroughly tested to ensure optimal performance.

IMPORTANT ! Do not freeze the magnetic particles.

4. Required equipment (not supplied as part of the kit)

- **Adem-Mag MODULO** (#20105, #20108) or **Adem-Mag 96** (#20106):
- Nuclease free microtubes or microplates
- Nuclease free tips
- Disposable gloves
- Thermal shaker or Heat block



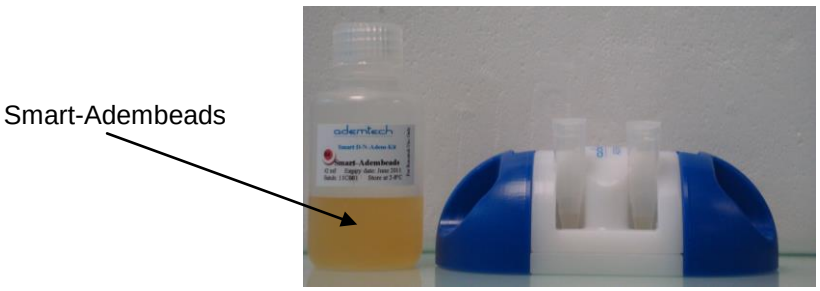


Smart D-N-Adem Kit Protocol

1. Smart-Adembeads Guidelines

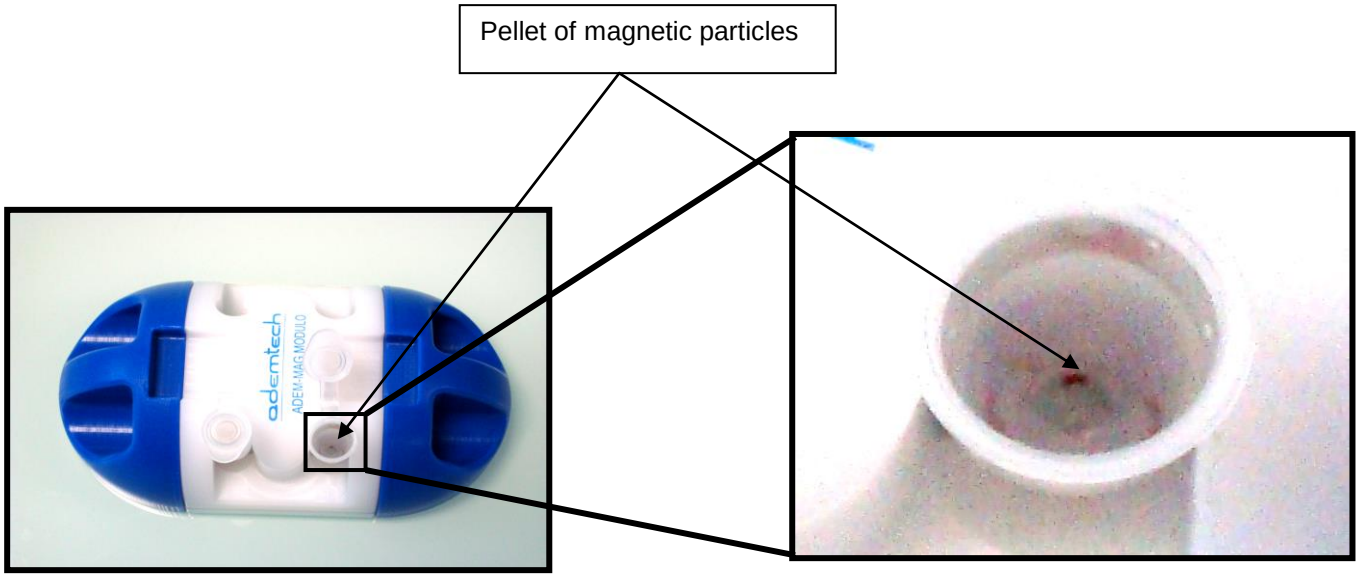
- Before using Smart-Adembeads, thoroughly flick / vortex the bottle to completely resuspend the magnetic particles.

- 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.



- During separation steps, let the microtubes containing magnetic particles on the magnet at least 3 minutes. Pay attention to the size of the magnetic pellet which is very small. The magnetic particles pellet is oriented toward the magnet at the back of the microtubes.

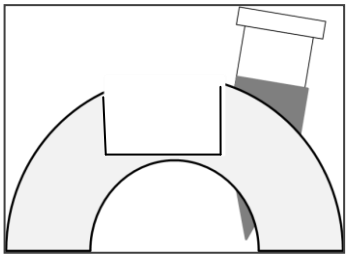
- When removing the liquid phase, pipette off carefully, do not aspirate magnetic particles or disturb the magnetic pellet.



2. Magnetic Stand Guidelines

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

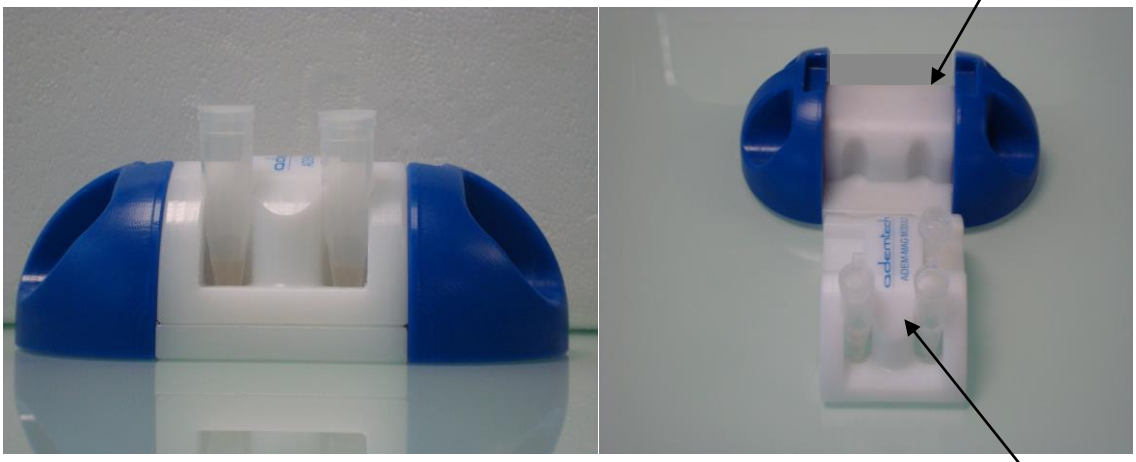
- Insert the microtubes into the sample holder in the correct position.



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. Smart D-N-Adem- Kit Protocol

3.1. Prepare samples

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 or a new microtube.
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.

3.2. Perform lysis

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 punches.

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.

3.3. Bind genomic DNA to magnetic particles for normalisation

1. Distribute **100µl of Smart-Adembeads** in a new microplate or a new microtube.
2. Transfer the lysate (supernatant) to the Smart-Adembeads.
3. Mix well by pipetting or vortex (during 5s, 1800-2000rpm).
4. Incubate at room temperature for 5 minutes at 900rpm.

NOTE! Increasing the incubation time with the Smart-Adembeads do not affect DNA yield.
Smart-Adembeads are ready to use (there is no need to add Binding Buffer).

3.4. Wash bound DNA

After binding DNA to the magnetic particles, wash the magnetic particles to remove impurities and inhibitors**.**

1. Place the tube or microplate on the magnet at least 5 minutes, and discard carefully the supernatant.
2. Remove the tube or microplate from the magnet and add **100µl of Washing Buffer** and mix by pipetting or vortex (during 15s, 900rpm).
3. Place the tube or microplate on the magnet for at least 5 minutes and discard the supernatant.

NOTE! After DNA binding, magnetic particles aggregates could be observed due to the binding of DNA onto the beads. Nevertheless, magnetic particles easily come back in suspension by mixing (pipetting or vortex) during washing step.

3.5. Elute DNA

After performing the washing step, resuspend the purified DNA and separate the DNA eluate from the magnetic particles.

1. Remove the tube or microplate from the magnet and add **80µl of Elution Buffer**.

IMPORTANT! Do not use water instead of Elution Buffer. Do not dry the magnetic particles

2. Place the tube or the microplate in a thermal shaker, then mix at room temperature at 900 rpm for 10 minutes.
3. Incubate overnight at 4°C the tube or the microplate. This step is to maximize DNA recovery.

NOTE! The isolated DNA may be stored at 4°C with the beads for up to one week or at -20°C for longer storage.

NOTE! An alternative elution procedure is to heat resuspended particles at 75°C, 900rpm for 5 minutes instead overnight at 4°C.

4. Place the tube or microplate on the magnet for at least 5 minutes and transfer carefully the liquid (supernatant) which contains the isolated genomic DNA to a new microtube of microplate.

When removing the liquid phase (supernatant), do not aspirate magnetic particles or disturb the magnetic particles pellet.

DNA analysis and expected results

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,
  - Perform Elution (3.5), step 1 through 3
  - Mix by pipetting or vortex and take an aliquot of 2µl for a 25µl PCR reaction.

For STR analysis

- For STR amplification and analysis, magnetic particles must be removed. Magnetic particles could migrate and interfere with subsequent analysis.
- For STR amplification, we recommended using between 3-7µl of DNA eluate.

Troubleshooting

Observations	Possible cause	SUGGESTION
Magnetic particles settled in the bottle.	During shipping, magnetic particles settle.	Thoroughly flick / vortex the bottle. Smart-adembeads are stored at 4°C, before using, incubate them at room temperature.
Supernatants contains 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 could disturb magnetic pellet	Keep the tube containing magnetic particles in the magnet for at least 5 minutes then pipette out carefully the supernatant Magnetic particles did not perturb DNA quantification but affect STR product migration for analysis
DNA eluate is colored	Insufficient washing for blood	Perform additional washes.
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
	Insufficient amount of magnetic particles added	

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.