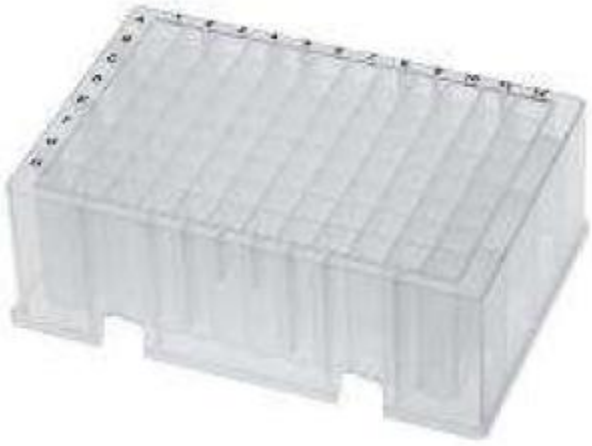
Kit for DNA extraction and normalization on
High throughput automated systems

Quick Overview

High-throughput extraction
Smart D-N-Adem-Kit
For Swab
1 x 100 (#06142)

For
Magnetic Handling Systems
&
Liquid Handling Systems



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Smart D-N-Adem-Kit for Swab (#06142)
on
High Throughput Systems
Quick Overview

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The Smart D-N-Adem-Kit for Swab

INTENDED USE

DNA quantities present on 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 for swab 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.

KIT CONTENT

Each kit contains enough reagent to perform 100 reactions using the following standard protocol. Kit contents are described in the table below.

Table 1: *Materials provided within Smart D-N-Adem-Kit for swab (# 06142)*

Smart D-N-Adem-Kit for Swab (#06142)			
	Amount	Reagents	Storage conditions
R1	50µl	RNase A	+ 2°C to +25°C
R2	250µl	Proteinase K	+ 2°C to +25°C
R3	50ml	Lysis Buffer	+ 2°C to +25°C
R4	10ml	Smart-Adembeads	+ 2°C to +25°C
R5	10ml	Washing Buffer	+ 2°C to +25°C
R6	10ml	Elution Buffer	+ 2°C to +25°C
contains sufficient reagents to perform **100 normalisations**			

STABILITY AND STORAGE CONDITIONS

The kits are sent at room temperature and stored at 4°C or room temperature upon reception.

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.

IMPORTANT! Do not freeze the magnetic particles.

CAUTIONS AND WARNING

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

MATERIALS AND REAGENTS NEEDED

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

Products needed but available separately :

- DeepWell Plate (Cat. #21102)
- 96-tip Comb (Cat. #21105)

Materials needed but not provided :

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

Magnetic Handling Systems KingFisher Flex

PRECAUTIONS

I) Before using

• Do not operate KingFisher Flex whitout qualify 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.

- **Sample preparation :**

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

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

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

- **DNA extraction on KingFisher Flex :**

1. Preparation of the 3 DeepWell plates with the volume indicated below (table) in each well

DW plate	Reagents	Volume / well
Elution	**Elution Buffer**	**65 µL**
Washing Buffer	**WB**	**100 µL**
Binding	**Smart-Adembeads**	**100 µL**
Tip Plate	**place a 96-tips comb in the DW plate**	

For automated pipetting:

1a- Place 3 x 60ml cuvettes and 4 x Deep Well plates on the pipetting machine.

1b- Pour Smart Adembeads into the first cuvette. Pour Washing Buffer into second cuvette. Pour the Elution Buffer into the third cuvette.

1c- Distribute each of the 3 buffers in a Deep Well plate according to the quantities described above in the table, according to the corresponding liquid class (see definition of liquid classes): "Elution" for the Elution Buffer and "Main" for the other reagents.

2. Add 60 µL of lysate to the Binding plate according to the "Main" liquid class (see definition of liquid classes).

3. Start the Smart for Profiling v2.0 (Flex) extraction program on the KingFisher Flex and follow the instructions, adding each plate to the corresponding rack.

4. Place the DW plate containing the lysate in the machine and start automated extraction.

5. At the end of the run, recover the elution plate containing the eluate and store it using appropriate storage conditions.

NOTE ! If DNA is not analyzed immediately, it can be stored at 4°C for up to 24 hours. For longer storage, consult local laboratory instructions. Samples stored at -20°C have been shown to last longer.

Steps data

Tip1		96 DW tip comb	
	Pick-Up	Tip-plate	
	Binding	Binding Plate	
	Beginning of step	Precollect	No
		Release time, speed	00:00:05, Fast
	Mixing / heating:	Mixing time, speed	00:05:00, Medium
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	30
	CollectBeads1	Binding Plate	
		Collect count	5
		Collect time [s]	30
	Washing	Washing Plate	
	Beginning of step	Precollect	No
		Release time, speed	00:00:05, Bottom mix
	Mixing / heating:	Mixing time, speed	00:00:30, Medium
		Heating during mixing	No
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	30
	CollectBeads2	Washing Plate	
		Collect count	5
		Collect time [s]	30
	Elution	Elution Plate	
	Beginning of step	Precollect	No
		Release time, speed	00:00:30, Bottom mix
	Mixing / heating:	Mixing time, speed	00:05:00, Bottom mix
		Heating temperature [°C]	75
		Preheat	Yes
	End of step	Postmix	No
		Collect count	5
		Collect time [s]	30
	CollectBeads3	Elution Plate	
		Collect count	5
		Collect time [s]	30
	Leave	Tip-plate	

Magnetic Handling Systems KingFisher Presto

PRECAUTIONS

I) Before using

- Do not operate KingFisher Presto whitout qualified operation training.
- Please read this manual carefully before using the controller..

II) Handling requirements

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

III) Laboratory procedures

- Handle all samples and resulting waste as if they were potentially infectious, using safe laboratory procedures. As the sensitivity and titer of potential pathogens in the sample varies, the operator must optimize pathogen inactivation by the capture buffer or take appropriate measures in accordance with local safety regulations. Ademtech does not guarantee that samples treated with the capture buffer are completely inactivated and non-infectious. Once sample processing is complete, remove and autoclave all disposable plastics.
- Do not eat, drink or smoke in the laboratory work area.
- Wear disposable protective gloves, lab coats and goggles when handling samples and kit reagents.
- Do not use sharp objects when working with reagent cartridges, to avoid damage to the sealing film and loss of reagent.
- Do not contaminate reagents with bacteria, viruses or ribonucleases. Use disposable pipettes and RNase-free pipette tips only to remove aliquots from reagent vials.
- Follow the general precautions described in the literature.
- Wash your hands thoroughly after handling samples and testing 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 reactions (#21102)	4	
96-tips comb for DW96 (#21105)	1	
Rack 16 tubes	13	
Cuvette (25 ml)	4	
Filter tips (200 µL)	8	

PROTOCOL

Read the complete protocol carefully before starting.

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

Good laboratory practice is essential.

The standard protocol is suitable for extraction on swab.

3. **Sample preparation :**

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

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

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

- **<u>DNA extrcation on KingFisher Presto</u> :**

- Installation of materials and reagents (cuvettes, buffers, tubes, racks)
- Distribution of buffers in the various DWs according to the table below

DW plate	Reagents	Volume / well
Elution	**Elution Buffer**	**65 µL**
Washing Buffer	**WB**	**100 µL**
Binding	**Smart-Adembeads**	**100 µL**
Tip Plate	**place a 96-tips comb in the DW plate**	

In the case of automated pipetting :

a- Place 3 x 60ml cuvettes and 4 x Deep Well plates on the pipetting machine

b- Pour Smart Adembeads into the first cuvette. Pour Washing Buffer into second cuvette. Pour the Elution Buffer into the third cuvette.

c- Distribute each of the 3 buffers in a Deep Well plate in the quantities described above in the table according to the corresponding liquid class (see definition of liquid classes): "Elution" for the Elution Buffer and "Main" for the other reagents.

- Lysate distribution in DW Binding (60 µL)

- Start the extraction process by following the same instructions as for programming the KingFisher Flex (see section "Programming the KingFisher Flex").
- Transfer eluates to collection tubes (60µL)
- For liquid classes, please refer to the paragraph "Definition of liquid classes".

Liquid Handling Systems (Hamilton, Tecan…)

MATERIAL NEEDED FOR LIQUID HANDLING SYSTEM (not provided)

Name	Quantity	Material
96-well magnetic rings	1	
Heater Shaker (3mm) and DeepWell adaptator	1	
Rack 16 tubes	13	
Cuvette (25 ml)	4	
Filter tips (200 µL)	10	
Deepwell plate for 96 reactions (#21102)	1	

PROTOCOL

Read the complete protocol carefully before starting.

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

Good laboratory practice is essential.

The standard protocol is suitable for extraction on swab.

4. **Sample preparation :**

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

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

1. Place the extremity of the dry swab in a 2mL microtube.
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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.
4. After the incubation, mix vigorously for 5 seconds.
5. Use only **50µl of lysat** to perform DNA extraction.

- **<u>Extraction on liquid handling system</u>:**

1 Initialization

- Installation of materials and reagents (buffers, tubes, racks)
- Transfer Deepwell 96 (DW96) plate to heater/shaker (HSS)
- Homogenization of "Smart-Adembeads" magnetic particles (200µL mix 5 times)
- Distribution of Smart-Adembeads in DW96 (100 µL)

2 Capture

- HSS : Lysate distribution in DW96 (60 µL)
- HSS : 600 sec, 800 rpm, TA (3x200 sec / clockwise (CW) / counterclockwise (CCW))
- Transferring the DW96 to the magnet
- Magnet : 300 sec
- Magnet : Supernatant removal (180 µL suction)
- Transfer DW96 on HSS

3 Washing

- HSS : Distribution of WBI in DW96 (100 µL)
- HSS : 60 sec, 800 rpm, RT (2x30 sec, CW/CCW)
- DW96 transfer to magnet
- Magnet : 300 sec
- Magnet : Supernatant removal (120 µL suction)
- Transfer DW96 on HSS

4 Elution

- *Set the HSS temperature to 75°C*
- HSS : Elution buffer distribution (65 µL)
- HSS : 300 sec, 800 rpm, 75°C
- Transfer DW96 on magnet
- Magnet : 300 sec
- Magnet : Transfer eluates into collection tubes (60µL)

Liquid Class Definition

Liquid Class	Range	Movement	Speed	Position	Offset	Liquid Detection	Note
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 compliance to the instructions in the operating instructions is necessary to ensure optimum results.

Ordering informations

- **Kits Ademtech**

CAT NO.	PRODUCT	FORMAT
06140	Smart D-N-Adem-Kit for Profiling	100 tests
21102	DeepWell plate	50 pc
21105	96 tips Comb	100 pc