Smart D-N-Adem-Kit

For Bacteria
(Gram-positive and Gram-negative)

(cat #06131)

Instruction manual for
gDNA capture and direct amplification

- **Bacterial Culture**
- **Individual Colonies**

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General Overview

Biomagnetic separation technology is a simple technique based on the separation **of superparamagnetic beads** using a magnetic field. When added to a complex medium, the magnetic particles will bind to the target. This interaction is based on the specific affinity of the ligand to the surface of the beads. The resulting target-bead complex can be removed from the suspension using a magnet. The inherent benefits of magnetic handling allow for easy washing, separation and concentration of the target without any need of centrifugation or columns.

Superparamagnetic beads exhibit magnetic properties only when placed within a magnetic field and show no residual magnetism when removed from this field.

Smart D-N-Adem-Kit for Bacteria

1. Product Description

1.1. General Description

The Smart D-N-Adem-Kit is a new system which is especially designed for capture of gDNA and direct amplification on magnetic beads. Smart-Adembeads have specific and proprietary polymer on their surface, designed for DNA capture by electrostatic interactions and compatible with direct PCR and Real-Time PCR without the need to perform an elution step.

Bacteria are mixed with the Lysis Buffer and Enzymatic Digestion Cocktail. Smart-Adembeads bind specifically to the gDNA. Proteins and other contaminants are then eliminated in the washing step. The purified gDNA bound to the Smart-Adembeads can be used directly for PCR and Real-Time PCR analyses. DNA isolation is achieved without phenol, ethanol, chloroform and ionic chaotropes; thus the purified gDNA bound to the Smart-Adembeads demonstrates improved downstream performance in PCR and Real-Time PCR. Unlike other purification systems, no elution of the gDNA is required, making it possible to maximize the amount of templates available for the reaction achieving greater sensitivity. This method makes this Kit ideal for processing small amount of samples where maximun gDNA recovery is critical.

1.2. Kit capacities

Sample	Amount of starting material	Number of isolations
Bacterial culture	Until 10µl	50 isolations
Individual colonies from an agar plate	1 colony	50 isolations

Table 1: *Number of isolation per amount of starting material*

1.3. Reagents provided with the kit

The Smart D-N-Adem-Kit includes reagents for performing 50 gDNA isolations. The provided reagents are listed below.

	Amount	Component	Storage
R1	250 µl	Smart-Adembeads	+ 4°C
R2	50 µl	RNase A (2mg/ml)	+ 4°C
R3	250 µl	Proteinase K (10mg/ml)	+ 4°C
R4	5 ml	Lysis Buffer	+ 4°C
R5	5 ml	Binding Buffer	+ 4°C
R6	10 ml	Washing Buffer	+ 4°C
R7	2.5 ml	Amplification Buffer	+ 4°C
R8	Freeze dried	Enzymatic Digestion Cocktail	+ 4°C

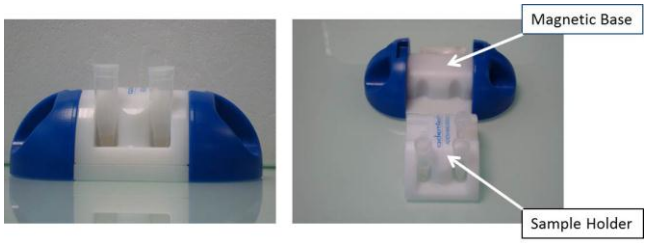
Table 2: *Reagent provided with the kit*

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

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

- Magnets : Adem-Mag SV (#20101) or Adem-Mag MODULO (#20105):

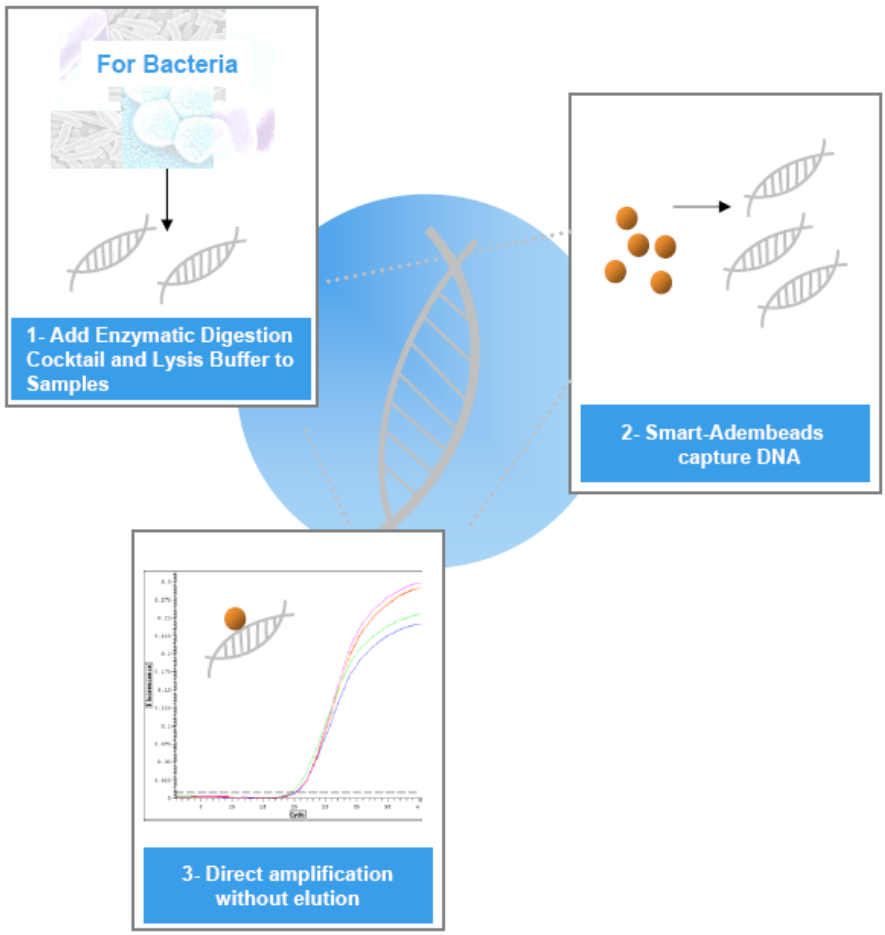
Adem-Mag MODULO



- Nuclease free microtubes
- Nuclease free tips
- Disposable gloves

2. Smart D-N-Adem-Kit Protocol

2.1. Smart D-N-Adem-Kit procedure



2.2. General Guidelines

- Smart D-N-Adem-Kit is only suitable for capture and direct amplification of gDNA on magnetic beads from small amounts of samples without the need for an elution step.
- Using UV spectrophotometric measurements (A260/A280 nm), is not recommended as this method is inaccurate for low DNA.
- Before starting gDNA extraction carefully read the paragraph 2.3.6.
- Before starting gDNA extraction, all buffers should be at room temperature to ensure optimal performances.

2.3. Protocol for gDNA isolation from bacterial culture or individual colonies picked from an agar plate

Enzymatic Digestion Cocktail Preparation

Add 265µl of Nuclease Free Water. Divide it into conveniently sized aliquots according to your utilization in order to avoid freeze-thawing cycles.

 The aliquots of Enzymatic Digestion Cocktail solution must be stored at -20°C for long term storage.

2.3.1 Sample Preparation

<u>From bacterial culture</u>: Procedure described up to 10µl

1. Transfer 10µl of bacterial culture to a 1.5ml microcentrifuge tube.
2. Add 50µl of Lysis Buffer (see Table 4, page 10).
3. Add 5µl of Enzymatic Digestion Cocktail. Mix by pipetting (or flick the tube) and incubate at room temperature for recommended times (see Table 3, page 9).
4. Add 5µl of Proteinase K and 1µl of RNase. Mix by pipetting (or flick the tube) and incubate at room temperature for 5 minutes.
5. Go to paragraph **2.3.2**.

<u>Note:</u> Alternatively, you can harvest cells from up to 0.5 ml overnight bacterial culture by centrifugation (10000 rpm, 5 minutes) and resuspend the pellet directly with 50µl Lysis Buffer. Go to paragraph 2.3.1/3.

From individual colonies picked from an agar plate:

1- Picked up 1 colony bacteria from an agar plate and resuspend it in 50µl of Lysis Buffer (see Table 4, page 10).
2- Add 5µl of Enzymatic Digestion Cocktail. Mix by pipetting (or flick the tube) and incubate at room temperature for recommended times (see Table 3, page 9).
3- Add 5µl of Proteinase K and 1µl of RNase. Mix by pipetting (or flick the tube) and incubate at room temperature for 5 minutes.
4- Go to paragraph **2.3.2**.

Note:

- **Refer to Table 3 and Table 4 for the recommended amount of reagents and digestion time.**

2.3.2 DNA Capture

1- Add 5µl of homogenized Smart-Adembeads (see Table 4, page 10).
2- Add 50µl of Binding Buffer and homogenize by pipetting (see Table 4, page 10). Incubate at room temperature for 1 minute.

Note:

- **Do not increase the incubation time, DNA capture efficiency could decrease.**
- **To save time, a solution containing the Smart-Adembeads and Binding Buffer should be prepared to add directly to your lysis samples.**

2.3.3 Washing of [DNA Smart-Adembeads] complexes

1- Place the tube on the magnet for at least 1 minute or until supernatant clearing and discard the supernatant. Remove the tube from the magnet and add 100µl of Washing Buffer and mix by pipetting.
2- Repeat once step 1 using 100µl of Washing Buffer.

Note: When removing the supernatant, start by pipetting the potentially formed foam.

2.3.4 Resuspension of [DNA Smart-Adembeads] complexes

1- Place the tube on the magnet for at least 1 minute or until supernatant clearing and discard slowly the supernatant.

2- Resuspend beads in 50µl of Amplification Buffer and mix by pipetting (see Table 4, page 10).

Note: Formation of aggregates may occur, due to DNA capture by beads, which can be easily resuspended by pipetting.

3- Use the final solution directly for PCR and Real-Time PCR (see recommendations in paragraph **2.3.6**) or/and store it at -20°C (long-term storage).

2.3.5 Quick Procedure

Bacteria	Enzymatic digestion cocktail	Incubation time	Temperature
Gram-negative	5µl	5-15 minutes	Room temperature
Gram-positive			
Staphylococcus	5µl	5-15 minutes	Room temperature
Streptococcus	5µl	2 hours	Room temperature
Enterococcus	5µl	2 hours	Room temperature
Listeria	5µl	15 minutes	Room temperature

Table 3: *Enzymatic Lysis Conditions*

	Bacteria	Up to 10µl
R4	**Lysis Buffer**	50µl
R8	**Enzymatic Digestion Cocktail**	5µl
R3	**Proteinase K**	5µl
R2	**RNase**	1µl
R1	**Smart-Adembeads**	5µl
R5	**Binding Buffer**	50µl
R6	**Washing Buffer (x2)**	100µl
R7	**Amplification Buffer**	50µl

Table 4: *Buffer amounts for gDNA preparation*

2.3.6 Recommendations for direct DNA amplification on Smart-Adembeads

- **General guidelines for PCR**

1- Homogenize the final solution thoroughly (gDNA/Beads complexes in Amplification Buffer) and add the recommended volumes (see below) to the PCR mixtures.
2- Perform your PCR reactions following manufacturers directions.
3- Make sure the number of cycles is sufficient and the primers have been tested and are satisfactory quality for amplification of small quantities of DNA.

- **Performing Standard PCR:**

Using 1-5µl of the final solution (gDNA/Beads complexes in Amplification Buffer) is recommended for 25µl or 50µl of PCR reaction.

- **Performing Real-Time PCR for detection:**

Using 1-5µl of the final solution (gDNA/Beads complexes in Amplification Buffer) is recommended for a 25µl or 50µl of PCR reaction.

Troubleshooting

No or low amounts of PCR products detected after standard PCR or qPCR.

- **DNA degradation:** Ensure that the process is completed in a nuclease free environment to avoid introducing any nucleases during the procedure or later handling.

- **Optimize the enzymatic digestion step:**
  Incubation time should be increased if necessary.

- **Too much gDNA/beads complexes used as PCR templates:** Decrease the volume of the final solution (gDNA/beads in Amplification Buffer) that is introduced in PCR reactions.

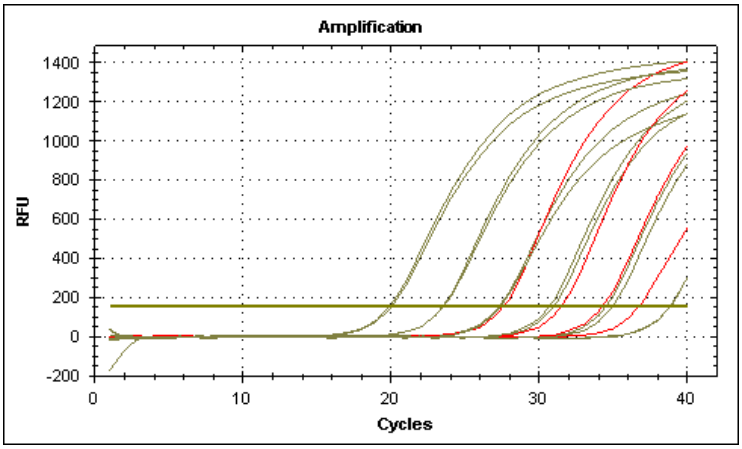
- **Optimize the qPCR or PCR assay:**
  - To improve results, increase the denaturation step (3-5 minutes).
  - Optimization parameters include primer design, primer concentration, probe design and probe concentration.
  - For best results, design primers that produce amplicons <150 bp in length.
  - Increase the number of PCR cycles.
  - Determine the optimal primer binding temperature for the PCR cycling program.
  - For long strength fragment, a more processive DNA polymerase is recommended.

Appendix: Determination of the yield of DNA bound onto Smart-Adembeads

- We do not recommend using UV spectrometric measurements (A260/A280 nm), this method is inaccurate for low DNA concentrations.

- Real-Time PCR is the most appropriate and precise approach for determining DNA yields. **Use a standard curve** that is prepared from a dilution series of a reference template. Prepare a standard dilution with a genomic reference DNA.

Note: Ideally a standard curve consists of at least 4 points, and each concentration should be run at least in duplicate.



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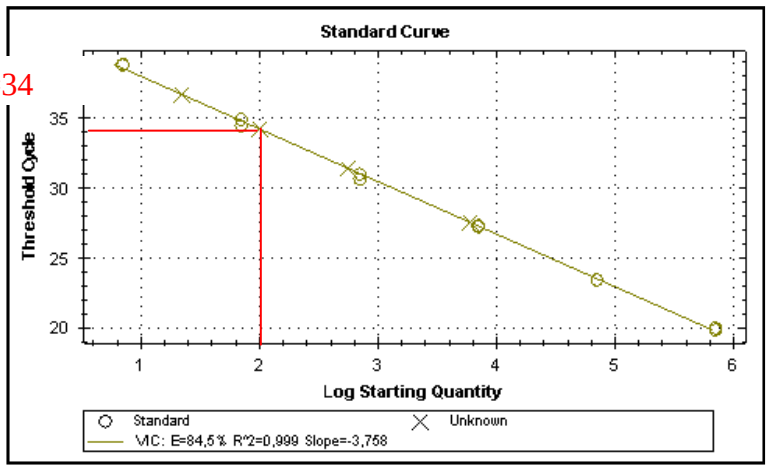

[DNA]=100ng

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.