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Smart D-N-Adem-Kit

For cells and animal tissues

(ADE-06121)

Instruction manual for gDNA capture and direct amplification

- **Cells**
- **Animal Tissues**

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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 Cells and Tissues

1. Product Description

1.1. General Description

The Smart D-N-Adem-Kit is a new system which is specially designed for the capture of gDNA and direct amplification on magnetic beads. Smart-Adembeads have a 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.

Animal tissues or cells are mixed with the Lysis Buffer. 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. The Smart D-N-Adem-Kit procedure allows cleaning gDNA in less than 10 minutes, lysis step included. 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 qPCR. Unlike other purification systems, no elution of the DNA is required, making it possible to maximize the amount of templates available for the reaction archieving greater sensitivity. This method makes this Kit ideal for processing small amount of samples where maximun DNA recovery is critical.

1.2. Kit capacities

Sample	Amount of starting material	Number of isolation
Cells	10- 5x10^5	100 isolations
Animal Tissues	Up to 25mg	100 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 100 gDNA isolations. The provided reagents are listed below.

	Amount	Component	Storage
R1	0.5ml	Smart-Adembeads	+ 4°C
R2	0.1 ml	RNase A (2mg/ml)	+ 4°C
R3	0.5 ml	Proteinase K (10mg/ml)	+ 4°C
R4	15ml	Lysis Buffer	+ 4°C
R5	15 ml	Binding Buffer	+ 4°C
R6	20ml	Washing Buffer	+ 4°C
R7	5 ml	Amplification Buffer	+ 4°C

Table 2: *Reagent provided with the kit*

Properly stored Kits are guaranteed until the expiration 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 performance.

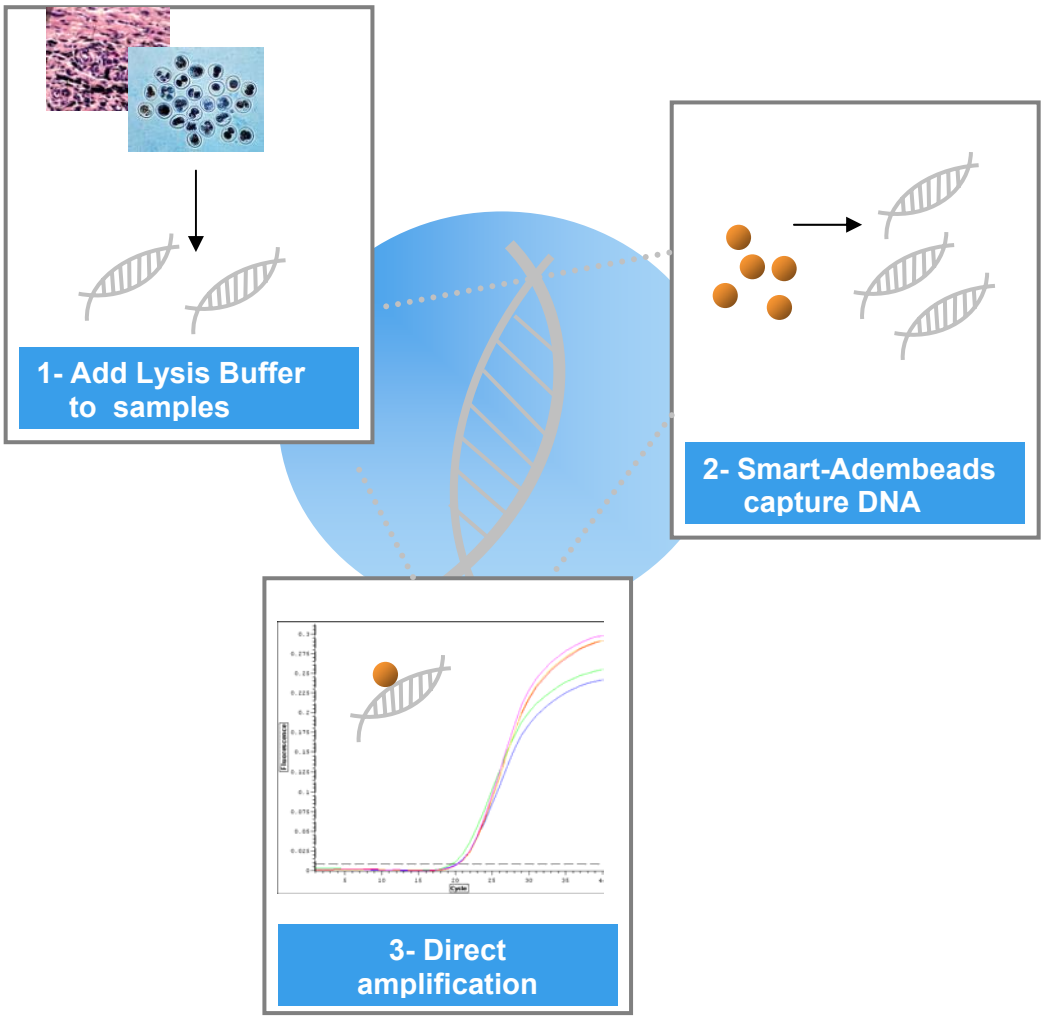
1.4. Required equipment (not supplied as part of the kit)

- Adem-Mag SV (#20101) or MSV (#20104):
- Nuclease free microtubes
- Nuclease free tips
- Disposable glove

Adem-Mag MSV

2. Smart D-N-Adem-Kit Protocol

2.1. Smart D-N-Adem-Kit procedure



2.2. General Guidelines

- The Smart D-N-Adem-Kit is suitable only 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 the gDNA extraction procedure, carefully read the paragraph **2.3.6**.
- Before starting the gDNA extraction procedure all buffers should be at room temperature to attain optimal performances.

2.3. Protocol for gDNA isolation from cells and animal tissues

2.3.1 Sample Preparation:

From cells: Procedure described up to 5x105 cells

1. Collect the cells and transfer them to a 1.5ml microcentrifuge tube in order to have them in a minimal volume of culture media or PBS (up to 1/5 of the Lysis Buffer volume, see Table 3).
2. Add 50µl to 150µl of Lysis Buffer (see Table 3).
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**.

From animal tissues: Procedure described up to 25mg

1- Add 100µl of Lysis Buffer and 5µl of Proteinase K to a 1.5ml microcentrifuge tube. Flick the tube (or mix by pipetting).
2- Immerse fresh or thawed tissues (up to 25mg) in the lysis solution and incubate at room temperature for 5-10 minutes

Note: Be careful not to dilacerate or vortex the tissue.

3- Remove and transfer the lysis solution to a new 1.5ml microcentrifuge tube.
4- Add 1µl of RNase. Flick the tube (or mix by pipetting) and incubate at room temperature for 5 minutes.
5- Go to paragraph **2.3.2**.

From rodent tail, ears and phalanx: Procedure described up to 0.5cm

1- Add 100µl of lysis buffer and 5µl Proteinase K to a 1.5ml microcentrifuge tube. Flick the tube (or mix by pipetting).
2- Immerse fresh or thawed tissues (up to 0.5cm) in the lysis solution and incubate at 65°C for 1 hour.

3- Remove and transfer the lysis solution to a new 1.5ml microcentrifuge tube.
4- Add 1µl of RNase. Flick the tube (or mix by pipetting) and incubate at room temperature for 5 minutes.
5- Go to paragraph **2.3.2**.

Note:

- **Refer to Table 3 and Table 4 for the recommended amount of reagents.**
- **To save time, a solution containing Lysis Buffer, Proteinase K and RNase can be prepared to add directly to the starting samples. Caution, RNase should be added in the last to avoid its early degradation by Proteinase K.**

2.3.2 DNA Capture

1- Add 1µl to 5µl of homogenized Smart-Adembeads (see Table 3 or Table 4).
2- Add 50µl to 150µl of Binding Buffer and homogenize by pipetting (see Table 3 or Table 4). Incubate at room temperature for 1 minute.

Note:

- **It is important not to increase the incubation time, that could decrease the efficiency of DNA capture.**
- **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 Wash [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 [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 10 to 50µl of Amplification Buffer and mix by pipetting (see Table 3 or Table 4).

Note: You may observe the formation of aggregates, 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 Choose your own starting point

Cells number	*10 - 10^2	10^2 – 25x10^3	25x10^3 - 10^5	10^5 – 5x10^5
Lysis Buffer	50µl	50µl	100µl	150µl
Proteinase K	5µl	5µl	5µl	5µl
RNase	1µl	1µl	1µl	1µl
Smart-Adembeads	**1µl**	**5µl**	**5µl**	**5µl**
Binding Buffer	50µl	50µl	100µl	150µl
Washing Buffer (x2)	100µl	100µl	100µl	100µl
Amplification Buffer	**10µl**	**50µl**	**50µl**	**50µl**

Table 3: Buffer amounts for DNA preparation from Cells

*For very small starting sample (less than 10 cells), resuspending the complexes DNA/beads in 5µl of amplification buffer and using all of the solution for the PCR detection is recommended.

Animal tissues	Up to 25mg	Tail, ears, phalanx
Lysis Buffer	100µl	100µl
Proteinase K	5µl	5µl
RNase	1µl	1µl
Smart Adembeads	**5µl**	**2µl**
Binding Buffer	100µl	100µl
Washing Buffer (x2)	100µl	100µl
Amplification Buffer	**50µl**	**20-50µl**

Table 4: Buffer amounts for DNA preparation from Tissues

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).
2- Add the volumes of the previous solution (see below) to the PCR mixtures.
3- Perform your PCR reactions following manufacturers directions.
4- 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:**

From cells and animal tissues, 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:**

From cells and animal tissues, using 1-5µl of the final solution (gDNA/Beads complexes in Amplification Buffer) is recommended for a 25µl of PCR reaction or 1-10µl of the final solution (gDNA/Beads complexes in Amplification Buffer) for a 50µl of PCR reaction.

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

From cells, the starting quantity of sample should not exceed $5x10^3$ cells. We recommend diluting your starting sample in PBS or culture media. Remenber, the diluted starting sample must not exceed 1/5 of the Lysis Buffer volume.

<u>To quantify DNA, the following volumes are recommended,</u>

*using 1-3µl of the final solution (gDNA/Beads complexes in Amplification Buffer) for a 25µl of PCR reaction.

*using 1-5µl of the final solution (gDNA/Beads complexes in Amplification Buffer) for a 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 lysis step:**
  From cells and animal tissues, incubation time should be increased an additional 5 or 10 minutes at room temperature.
  From rodent tail, ears and phalanx, incubation time should be increased until 30 or 60 minutes at 65°C.

- **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:**
  - For results improvement, the first PCR step (initial denaturation) could be increased at 3 to 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.

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.