Plant Prep Adem-Kit

(Cat #06220, cat #06221)

Instruction for manual protocol

ADEMTECH SA
Bioparc BioGalien
27, allée Charles Darwin 33600 PESSAC

France

Tel: +33557020201
Fax +3355702020

Visit our Web site: www.ademtech.com

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Introduction

1. Description

The Plant Prep Adem-Kit is specially designed for optimal DNA extraction from a broad panel of plant materiel. These samples include leafs, seeds, flour…

The DNA purified with the Plant Prep Adem-Kit is of high quality and free of proteins, nucleases, and other contaminants or inhibitors.

It is therefore suitable for direct use in many different downstream applications such as PCR (polymerase chain reaction).

This instruction manual also exists for automation protocol.

2. Product Component and storage conditions

Kit Content: Each Plant Prep Adem-Kit contains sufficient reagents to perform 96 samples using the following standard protocol.

Item	Plant Prep Adem-Kit
Cat No.	06220
Package size	1 x 96 samples
Plant Lysis Buffer	30mL
Prep-Adembeads	1.5mL
Washing Buffer I	24mL
Washing Buffer II	17mL
Elution Buffer	10mL
RNase	400µL

Plant Prep Adem-Kit (#06220)	
Reagents	**Storage condition**
Plant Lysis Buffer	+2-8°C
Prep-Adembeads	+2-8°C
Washing Buffer I	+2-8°C
Washing Buffer II	+2-8°C
Elution Buffer	+2-8°C
RNase	+2-8°C

Storage conditions: The kits are shipped at room temperature.

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.

NOTE 2! Storage conditions

All reagents in the kit can be stored at room temperature, except for the RNase which has to be stored at +2-8°C. This will not affect the stability.

For convenience, you can store the whole kit at +2-8°C. In this case, before using the kit, it is recommended to take out the reagents in advance and check if there are any precipitates. If the Buffers present precipitates place them at room temperature and eventually put them at +37°C.

IMPORTANT! Do not freeze the magnetic particles.

3. Equipment and reagents to be supplied by the user

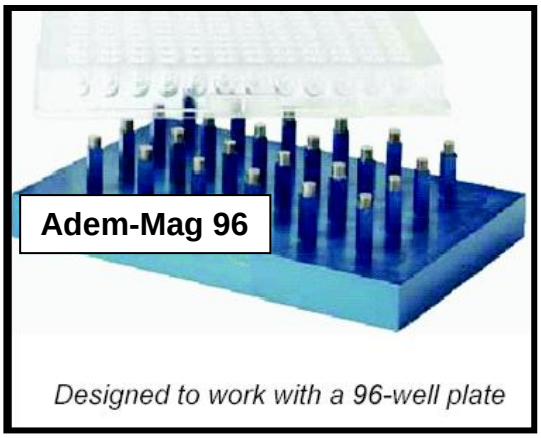
When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. To avoid contamination of your sample, wear face mask

Reagents:

- Isopropanol
- 96-100% ethanol
- 70% ethanol

Materials:

- 56°C heat block, thermomixer or water bath
- Microtubes or 96 Deepwell plates
- **Adem-Mag MODULO** (Cat.# 20105, # 20108) or **Adem-Mag 96** (Cat.# 20106)



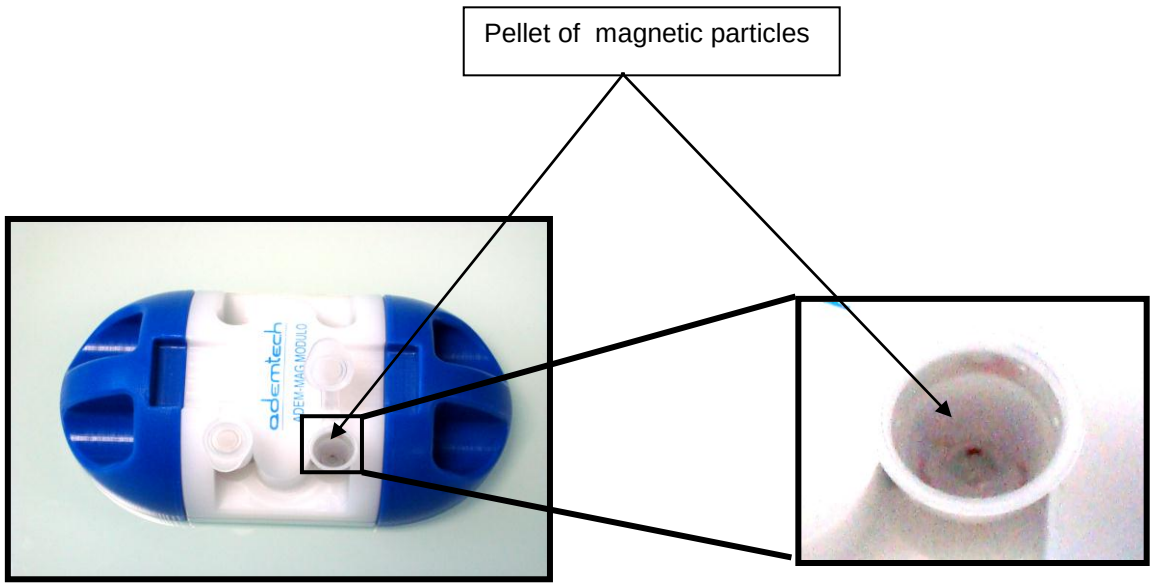
Plant Prep Adem-Kit Protocol

1. *Prep Adembeads Guidelines*

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

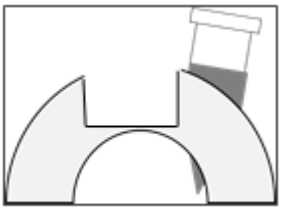
- During separation steps, let the microtubes containing magnetic particles on the magnet at least 3 minutes. The magnetic particles pellet is oriented toward the magnet at the back of the microtubes.

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



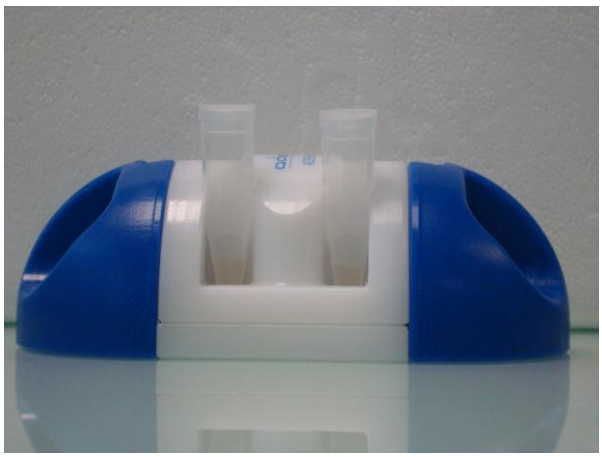
2. *Magnetic Stand Guidelines*

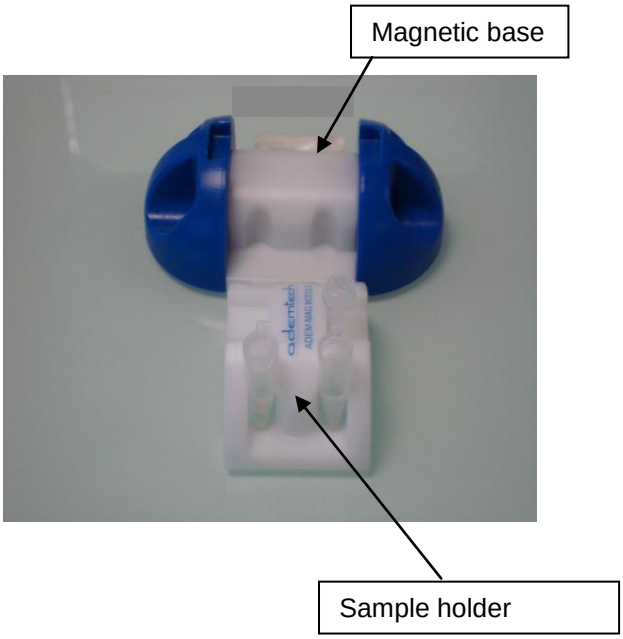
- Place magnetic base away from metal objects/magnetic media.
- Insert the microtubes into the sample holder in the correct position.



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. *Plant Prep Adem-Kit Protocol*

The standard protocol is appropriate for all kind of sample types, such as leafs, seeds, flour…

Plant Prep Adem-Kit is developed for the purification of DNA from ground plant material using paramagnetic particles.

samples		Recommended quantities
Maze, Wheat	**leaf**	2 -5 punches
	flour	~30mg
	seed	½ -1 seed
Sunflower	**leaf**	1 -2 punches
	seed	1 seed
Colza	**seed**	1 seed
	leaf	2-4 punches
Barley	**seed**	½ -1 seed
Lin	**seed**	1 seed

Recommended quantities of ground samples

3.1. Reagents preparation

- Prepare a 70% ethanol solution
- Prepare the Washing Buffers I & II before first use:
  1. Add 32mL of 96-100% ethanol to the bottle containing 24mL of Washing Buffer I. Homogenize the solution.
  2. Add 39mL of 96-100% ethanol to the bottle containing 17mL of Washing Buffer II. Homogenize the solution.

NOTE! Washing Buffer I & II are delivered concentrated. Before the first use, you have to **add ethanol** in the bottle in the indicated proportion.

3.2. Perform lysis

1. Place the ground sample at the bottom of a microtube.

2. Add 300µl of Plant Lysis Buffer to the microtube containing the ground sample.

3. Add 4µL of RNase.

4. Close the tube, mix well with a vortex and place it in a thermomixer, then incubate at +56°C and 1000rpm for 60minutes.

IMPORTANT! You can use a heat block or a water bath instead of a thermomixer.

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5. Centrifuge the lysat 20 minutes at 5600g.

6. Perform DNA extraction with 150µl of supernatant.

3.3. Bind genomic DNA

1. Transfer 150µL of clarified lysate in a new microplate or a new microtube.

2. Add 150µL of isopropanol and 15µL of Prep-Adembeads. Mix well by pipetting or by vortex.

NOTE! It is possible to prepare a premixed solution of Isopropanol / Prep-Adembeads.

Isopropanol	150µL	⎤	X number of extractions
Prep-Adembeads	15µL	⎦	

Add 165µL of premixed solution.

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3. Incubate at room temperature and 1000rpm for 5 minutes.

NOTE! During capture of DNA, it is important to shake in order to improve interactions between DNA and particles.

3.4. Wash bound DNA

After binding DNA to the magnetic particles, wash the magnetic particles to remove impurities and inhibitors. In this protocol, there are **three consecutives washes**.

1. **Washing I**

   a. Magnetize the particle suspension at least 5 minutes, and discard carefully the supernatant without disturbing the pellet of magnetic particle.

   b. Remove the microtube or microplate from the magnet and resuspend the pellet of magnetic particles in 500µL of Washing Buffer I.

2. **Washing II**

   a. Magnetize the particle suspension at least 5 minutes, and discard carefully the supernatant without disturbing the pellet of magnetic particle.

   b. Remove the microtube or microplate from the magnet and resuspend the pellet of magnetic particles in 500µL of Washing Buffer II.

3. **Washing III**

   a. Magnetize the particle suspension at least 5 minutes, and discard carefully the supernatant without disturbing the pellet of magnetic particles.

   b. Remove the microtube or microplate from the magnet and resuspend the pellet of magnetic particles in 500µL of 70% ethanol.

3.5. Drying

1. Magnetize the particle suspension at least 5 minutes.

2. Eliminate carefully the supernatant without disturbing the pellet of magnetic particles.

3. Let the pellet of magnetic particles dry for 5 minutes.

NOTE! Magnetization and Drying times are given as an indication.
The drying time may be reduced to facilitate the recovery of the pellet.

3.6. Elute DNA

1. Remove the microtube or microplate from the magnet and resuspend thoroughly the pellet of magnetic particles in 60-100µL of Elution Buffer.

IMPORTANT! Do not use water instead of Elution Buffer.

2. Incubate the microtube or microplate at 50°C and 1000rpm for 5 minutes.

3. Place the microtube or microplate on the magnet for at least 5 minutes.

4. Collect the supernatant containing pure DNA and transfer it to another microtube or microplate.

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.

Troubleshooting

Observations	Possible cause	SUGGESTION
Magnetic particles settled in the bottle.	During shipping, magnetic particles settled.	Thoroughly flick / vortex the bottle. Prep-Adembeads are stored between+2-8°C, before using incubate them at room temperature.
Supernatants contain 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 can disturb magnetic pellet	Keep the tube containing magnetic particles in the magnet for at least 5 minutes then pipette out carefully the supernatant
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 sample
	Insufficient amount of magnetic particles added	Review protocol steps and reagents additions

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.

Ordering Information

- **Ademtech Kits**

CAT NO.	PRODUCT	PACKAGE SIZE
06220	Plant Prep Adem-Kit	1 x 96
06221	Plant Prep Adem-Kit	6 x 96

- **Instrument and consumables**

CAT NO.	PRODUCT	PACKAGE SIZE
20105	Adem-Mag MODULO Classic	Each
20106	Adem-Mag 96	Each
20108	Adem-Mag MODULO Brick	Each