ChIP DNA Prep Adem-Kit

(Cat #06240)

Instruction for manual protocol
Instruction manual for automation protocol

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Introduction

1. Description

The ChIP DNA Prep Adem-Kit is specifically optimized for extracting DNA from Chromatin Immunoprecipitation.

The use of magnetic beads allows for a clear separation of DNA and increases the reproducibility of your DNA purification.

2. Product Component and storage conditions

Kit Content: Each ChIP DNA Prep Adem-Kit contains sufficient reagent to perform 100 samples using the following standard protocol.

Item	ChIP Prep Adem-Kit
Cat No.	06240
Package size	1 x 100 samples
LB Buffer	20mL
Prep-Adembeads	1.5mL
ChIP DNA Washing Buffer	2X 40mL
Elution Buffer	10mL

ChIP Prep Adem-Kit (#0000)	
Reagents	**Storage condition**
LB Buffer	+2-8°C
Prep-Adembeads	+2-8°C
CHiP DNA Washing Buffer	+2-8°C
Elution Buffer	+2-8°C

Storage conditions: The kits are shipped at 4°C.

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.

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.

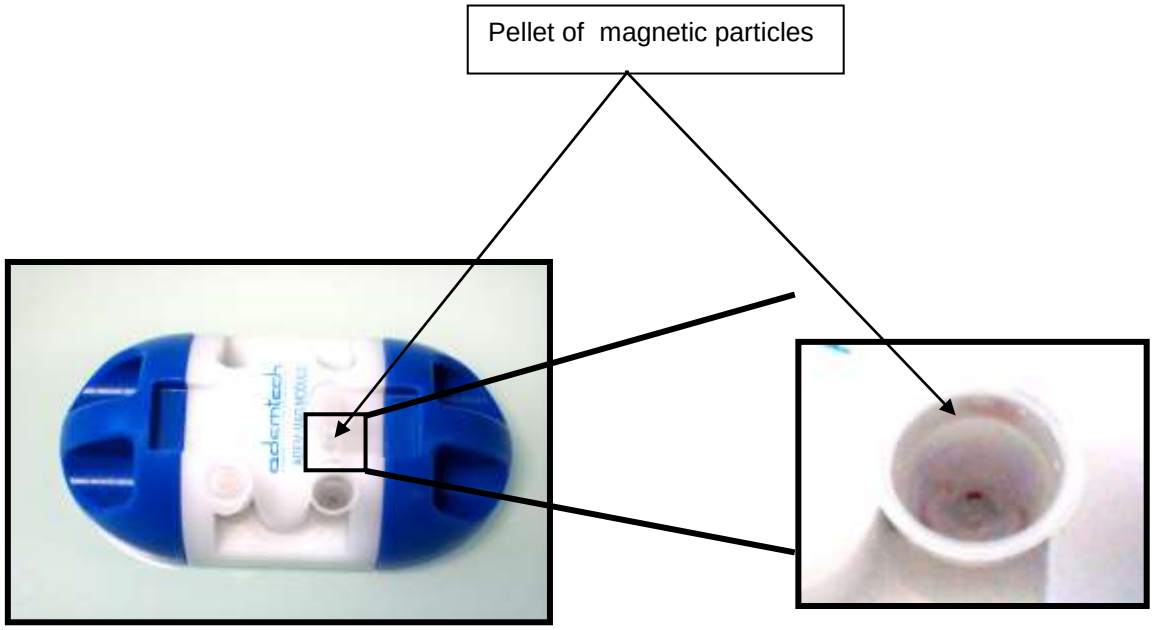
ChIP DNA Prep Adem-Kit Protocol

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 5 minutes. The magnetic particles pellet is oriented toward the magnet at the back of the microtubes.

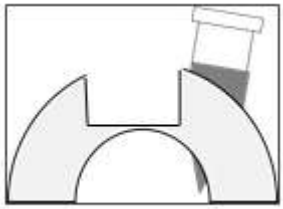
- When removing the liquid phase, pipette off carefully, do not aspirate magnetic particles or disturb the magnetic pellet



2. *Magnetic Stand Guidelines*

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

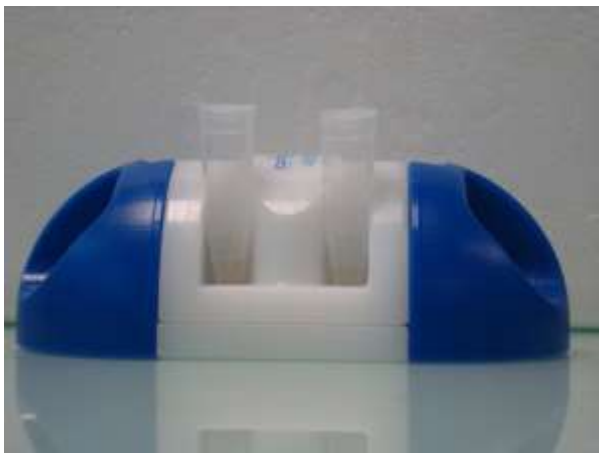
- Insert the microtubes into the sample holder in the correct position.

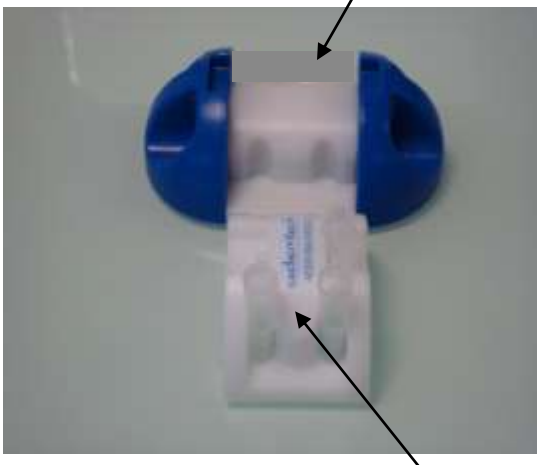


Correct position

- 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. 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

Materials:

- Thermomixer Mix-Heat
- Microtubes
- **Adem-Mag MODULO** (Cat.# 20105, # 20108)

Adem-Mag MODULO

Mix-Heat

4. *ChIP DNA Prep Adem-Kit Protocol*

4.1 Bind genomic DNA

1. Transfer 200µL of reverse chromatin in a new microtube.
Add **200µL of LB Buffer**. Mix well by pipetting.

 **WARNING ! CHEMICAL HAZARD. LB Buffer** in contact with acids or bleach liberates toxic gazes. Harmful if inhaled, absorbed through the skin, and swallowed. Cause eye, skin, and respiratory tract irritation. DO NOT ADD acids or bleach to any liquid wastes containing this product. Avoid breathing vapour. Do not taste or swallow. Use with adequate ventilation. Avoid contact with eyes and skin. Read the MSDS, and follow and handling instructions. Wear appropriate protecte eyewear, clothing and gloves.

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2. Add **200µL of isopropanol** and **15µL of Prep-Adembeads**. Mix well by pipetting.

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

Isopropanol	200µL	⎤	X number of extractions
Prep-Adembeads	15µL	⎦	

Add 215µL of premixed solution.

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3. Incubate at room temperature and 1000rpm for 10 minutes.

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

4.2 Wash bound DNA

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

1. Washing N°1

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 from the magnet and resuspend the pellet of magnetic particles in **500µL of ChIP DNA Washing Buffer**

2. Washing N°2

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 from the magnet and resuspend the pellet of magnetic particles in **250µL of ChIP DNA Washing Buffer**

4.3 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.

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4. Remove the liquid remaining in the bottom of the tube by pipetting

4.4 Elute DNA

1. Remove the microtube from the magnet and resuspend thoroughly the pellet of magnetic particles in **60µL to 100µl of Elution Buffer**.
2. Incubate the microtube at 50°C and 1000rpm for 5 minutes.
3. Place the microtube on the magnet for at least 5 minutes.
4. Collect the supernatant containing pure DNA and transfer it to another microtube.

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.

ChIP DNA Prep Adem-kit is compatible for Traditional PCR, Real-Time PCR, Digital PCR and Sequencing applications.

ChIP DNA Prep Adem-Kit Protocol for AutoMag

1. *AutoMag description*

AutoMag Instrument is a low-to-medium-throughput purification system with a magnetic particle processor for DNA purification kits. With the AutoMag Instrument customers can process up to 12 samples per run with a working volume up to 1 ml. In addition, it is possible to run two purification methods sequentially without interruption, raising the throughput up to 24 samples.

AutoMag Instrument (Cat.# 21106)

2. *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

Materials:

- Microtubes
- AutoMag Instrument (Cat.# 21106)
- Microtiter Deep well 96 plate (Cat.# 21102)
- 12-tip comb for 96 Deepwell plate (Cat.# 21103)
- Elution strip 40 pcs (Cat. # 21104)
- Cap for Elution strip 40 pcs/box (Cat.#21110)

3. *Instruction for DNA ChIP purification*

These instructions are for the DNA ChIP purification from 200µl of reverse DNA using the ChIP Prep Adem-Kit (Cat.# 06210) and the AutoMag Instrument (Cat.# 21106) with a 12−pin magnet head and 96 deep well plates.

Users can carry out the Elution step following two protocols:

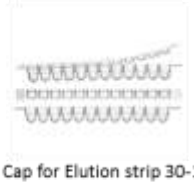
- Elution step in the 96 deep Well Plates (in Row A)
- Elution step in the Elution Strip

Instructions:

1. **Take one empty Microtiter Deep well 96 plate and an empty Elution Strip if Elution step is going to perform inside**

Microtiter Deepwell 96 plate (Cat#21102)

Elution strip 30-130µl and its support (Cat#????)



Cap for Elution strip 30-130µl (Cat#????)

3

2. Prepare the plate

Add the following reagents to the rows. ***Note that row B is reserved for the tip comb and should be left empty****. Note that row G and H are left empty. Note that row A is left empty if the Elution step is carry out in the Elution strip.*

Plate name and type	Row	Row name	Content	Reagent/Sample volume per well
Microtiter Deep well 96 plate	A	Elution	Elution Buffer	60µL
	B	Tip	12-tip comb	Empty
	C	Magnetic Particles	Prep-Adembeads **dilution 1:20**	300µL
	D	Binding	LB Buffer Isopropanol Sample	200µL 200µL 200µL
	E	Washing 1	ChIP DNA Washing Buffer	500µL
	F	Washing 2	ChIP DNA Washing Buffer	250µL
	G	Empty	Empty	Empty
	H	Empty	Empty	Empty

If Elution step is perform in the Elution strip, please add the following reagent to the row:

Plate name and type	Row	Row name	Content	Reagent/Sample volume per well
Elution strip	-	Elution	Elution Buffer	60µL

NOTE! It is possible to prepare a premixed solution of dilute Prep-Adembeads

Milli-Q Water	285µL	]	X number of extractions
Prep-Adembeads	15µL		

WARNING ! CHEMICAL HAZARD. LB Buffer in contact with acids or bleach liberates toxic gazes. Harmful if inhaled, absorbed through the skin, and swallowed. Cause eye, skin, and respiratory tract irritation. DO NOT ADD acids or bleach to any liquid wastes containing this product. Avoid breathing vapour. Do not taste or swallow. Use with adequate ventilation. Avoid contact with eyes and skin. Read the MSDS, and follow and handling instructions. Wear appropriate protecte eyewear, clothing and gloves.

Transfer **200µl of your reverse chromatin** in each well of the **Row D**

3. **Place a 12-tip comb for 96 Deepwell plate into row B of the plate.**

12-tip comb for 96 Deewell plate (Cat.# 21103)

4. **Start the ChIP Prep protocol with the AutoMag Instrument and load the plate**

   *Switch ON the AutoMag Instrument and make sure that you are using the 12-pin magnet head and heating block. Start the ChIP Prep protocol (**PurifChIPDW v1.2** if Elution step is carried out in Deepwell plate or **PurifChIPstrip v1.1** if Elution step is carried out in Elution strip). Insert the plate into the instrument as indicated on the AutoMag Instrument display and press **OK**.*

5. **After the run is completed, remove the plate and store the purified DNA-ChIP.**

   When the protocol is completed, remove the plate according to the instructions on the AutoMag Instrument display and turn off the instrument.

   If the Elution step was carry out in the Elution strip, please close the strip with its cap.

NOTE! Store or analyze the purified DNA-ChIP 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.

ChIP DNA Prep Adem-kit is compatible for Traditional PCR, Real-Time PCR, Digital PCR and Sequencing applications.

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

Observations	Comments and suggestions	
Error message in instrument display	Refer to the user manual supplied with your AutoMag Instrument.	
The tip comb holder lifting mechanism is out of position	Switch the instrument OFF and ON, and try again. If the error appears during initialization or is otherwise repeated, contact service.	
The turntable rotating mechanism is out of position	Switch the instrument OFF and ON, and try again. If the error appears during initialization or is otherwise repeated, contact service.	
The magnet head holder lifting mechanism is out of position	Switch the instrument OFF and ON, and try again. If the error appears during initialization or is otherwise repeated, contact service.	
The plastic tip comb is not attached to the holder	Check if the tips are presents. If it looks all right, turn ON and OFF, and run the check protocol.	
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

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.

Symbols

Reference Number

Symbol for batch code/lot number.

The symbol is accompanied by the manufacturer's batch code

Manufacturer

This symbol is accompanied by the name and address of the manufacturer.

Expiration Date

This symbol is accompanied by a date to indicate that the device should not be used after the end of the year, month or day shown.

Sufficient For

The number of items for which the contents of the pack is sufficient appears adjacent to the symbol.

Temperature Limitation / Temperature Range

Symbol for temperature limitation or temperature range. Both upper and lower limits are indicated adjacent to horizontal lines.

Ordering Information

- **Ademtech Kits**

CAT NO.	PRODUCT	PACKAGE SIZE
04243	ChIP Adem Kit Protein A	25 ChIP
04343	ChIP Adem Kit Protein G	25 ChIP
04242	ChIP Adembeads Protein A	40 CHIP
04342	ChIP Adembeads Protein G	40 CHIP

- **Instruments**

CAT NO.	PRODUCT	PACKAGE SIZE
20105	Adem-Mag MODULO Classic	Each
20106	Adem-Mag 96	Each
20108	Adem-Mag MODULO Brick	Each
21200	Mix-Heat	Each
21106	AutoMag Instrument	Each
21102	Microtiter Deepwell 96 plate	50 pcs
21103	12-tip comb for 96 Deepwell plate	50 pcs
21104	Elution Strip	40 pcs
21110	Cap for elution strip	40 pcs