



**ChIP-Adem-Kit**

**AutoMag Solution**

**(Cat #04643, #04644)**

**Instruction manual for automation protocol**

ADEMTECH SA  
Bioparc BioGalien  
27, allée Charles Darwin  
33600 Pessac

France

Tel: +33557020201  
Fax +3355702020

Visit our Web site: [www.ademtech.com](http://www.ademtech.com)

## ChIP Adem-Kit – AutoMag Solution

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## General overview

### 1. 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 their 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.

### 2. Product principle

A central element of genome function is the expression of the individual genome elements and the factors that regulate those events. Transcription factors, histone proteins that interact with DNA are critical players within the regulation of gene expression events.

**Chromatin Immunoprecipitation (ChIP)** assay, is an experimental method used in molecular biology to determine whether proteins including transcription factors or histones bind to a particular region on the endogenous chromatin. In outline, the method consists of the following steps. The protein under study is crosslinked to DNA which is subsequently extracted and sheared into approximately 0.2-1kb fragments. Whole Protein-DNA complexes can be immunoprecipitated using an antibody specific for the targeted protein. The DNA from the isolated Protein / DNA fraction can then be purified. The identity of the DNA fragments isolated in complex with the protein of interest can then be determined by PCR or Real-Time PCR using specific primers for the DNA regions that the protein in question is hypothesized to bind.

## ChIP Adem-Kit Protocol

### 1. Product description

#### 1.1 General description:

The ChIP-Adem-Kit is an innovative system especially designed for monitoring transcription factors or histones / DNA interactions.

MasterIP are uniform sized superparamagnetic particles (500nm) conjugated with protein AG. Protein AG is a genetically-engineered protein that combines the IgG binding domains of both Protein A and G. Recombinant fusion protein AG contains five Ig-binding regions of protein A and three Ig-binding. Protein AG binds to all IgG subclasses from various mammalian species, including all IgGs that bind both Protein A and Protein G, making the ideal choice for purification of all kinds of polyclonal and monoclonal IgG antibodies. The protein AG lacks albumin, cell wall binding regions and others non-specific binding regions to ensure maximum specific IgG binding. MasterIP after incubation with the Blocking Buffer have a specific surface designed for efficient Protein / DNA complex immunoprecipitation and to minimize non-specific enrichment. Proteins and other contaminants are eliminated in the washing steps. The ChIP protocol is greatly simplified by using blocked Protein recombinant A/G-coated nanoparticles. Some steps have been completely eliminated. Unlike traditional agarose beads, chromatin pre-clearing is not required. Master IP combined to especially designed buffers reduce background and maximize the amount of templates available for the reaction, achieving greater sensitivity where maximum DNA recovery is critical. The isolated DNA can be used in downstream applications : PCR and Real-Time PCR.

The ChIP-Adem-Kit contains all the buffers and reagents necessary to perform 24 chromatin immunoprecipitations. The protocol is optimized for use with mammalian cells

## ChIP Adem-Kit – AutoMag Solution

### 1.2 Reagents provided with the kit:

ChIP-Adem-Kit for AutoMag Solution (cat=04643)			
	Amount	Component	Storage
R1	13 ml	Lysis Buffer I	+ 4°C
R2	25 ml	Glycine Buffer	+ 4°C
R3	13 ml	Lysis Buffer II	+ 4°C
R4	3 ml	Lysis Buffer III	+ 4°C
R5	27ml	IP Buffer	+ 4°C
R6	1.2 ml	Master IP	+ 4°C
R10	60ml	Washing Buffer I	+ 4°C
R11&R12	3,8ml	Elution Buffer	+ 4°C
R13	250µl	Proteinase K (10mg/ml)	+ 4°C
R14	12,5µl	Protease Inhibitor Cocktail	+ 4°C
R15	6ml	<b><u>Blocking Buffer</u></b>	<b><u>+ 20°C</u></b>



The blocking Buffer must be stored at room temperature. Properly stored kits are guaranteed until the expiry date. Note that the shipping is realized at room temperature which will not affect its stability.

ChIP-Master Ip for AutoMag Solution (cat=04644)			
	Amount	Component	Storage
R1	5 ml	Master IP	+ 4°C
R2	25 ml	<b><u>Blocking Buffer</u></b>	<b><u>+ 20°C</u></b>

### 1.3 Required equipment to be supplied by the user:

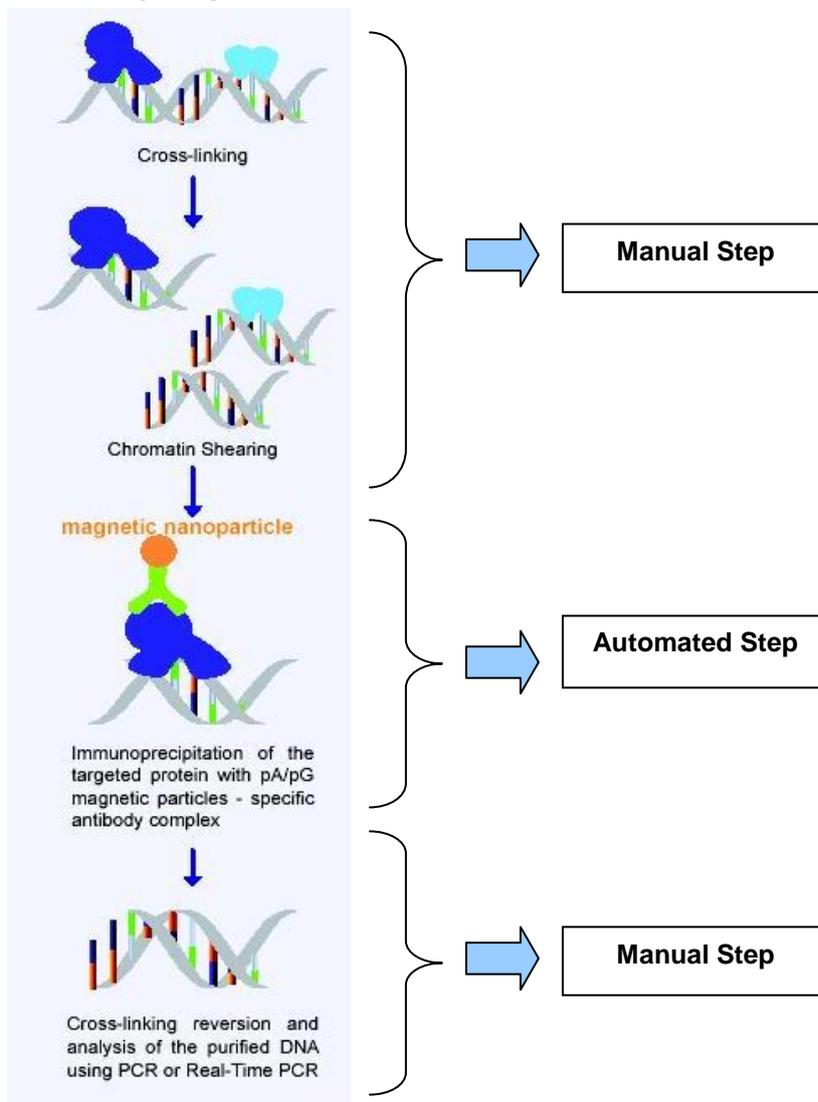
- Instrument for AutoMag Solution (Cat.# 21106)
- Antibodies of interest for chromatin immunoprecipitation
- 37% Formaldehyde
- Sonicator Thermal shaker or Heat block
- Disposable gloves
- Shaking platform
- Thermomixer (MixHeat Thermo Shaker # 21200)
- ChIP DNA Prep Adem-kit manual (#06240)
- ChIP DNA Prep Adem-kit Automag Solution (#06241)



Instrument for AutoMag Solution (Cat.# 21106)

## 2. ChIP protocol

### 2.1 Magnetic Immunoprecipitation Chromatin Procedure:



### 2.2 General Guidelines:

- The ChIP-Adem-Kit is suitable for studying the protein / DNA interactions and combines the specificity of the immunoprecipitation with qualitative or quantitative PCR.
- The method requires high quality antibodies capable of recognizing fixed protein that is bound to DNA and/or complexed with other proteins.
- Before starting, you must optimize sonication conditions to shear your DNA (200-1000bp), carefully read the paragraph 2.3.2.
- Blocking Buffer must be stored at room temperature. Lysis Buffer III and Elution Buffer contain SDS, warm these buffers to room temperature to ensure SDS is in solution before processing.
- Formaldehyde is defined as toxic and should be used in a ventilated fume hood. Appropriate safety precautions (gloves, safety glasses...) should also be taken.

Formaldehyde		Toxic
Risk Statements	H301+H311+H331 H314, H317, H335, H341, H350, H370	
Safety Statements	P201, P260, P280 P301+P310+P330 P303+P361+P353 P304+P340+P310 P305+P351+P338 P308+P311 P403+P233	

- Please read the entire protocol before starting

### 2.3 Protocol for ChIP:

#### 2.3.1 Cell collection and *In Vivo* crosslinking



Crosslinking is a critical step that must be optimized.

Advice on crosslinking

- It is important to optimize the fixation step by testing different formaldehyde concentrations (1-3%) and different incubation times (5-30 min).
- Efficiencies of chromatin shearing and immunoprecipitation of the targeted protein are linked to the crosslinking process.
- Longer crosslinking times and / or higher formaldehyde concentrations may decrease shearing efficiency but could improve immunoprecipitation of proteins that are not directly bound to DNA.
- Use high quality formaldehyde.
- Histones are tightly linked to DNA, crosslinking step is optional.
- To obtain a good reproducibility, we recommend to culture a constant and well known number of cells 24 h before your experiment.

**The protocol from point 1 to 5 is done for a single culture plate = one ChIP test.**

1. Cells (treated or untreated) are grown to 60%-80% confluence on a 100mm plate ( $2-4 \times 10^6$  cells/plate).
2. Remove as much cell medium as possible. Cross link proteins to DNA by adding 10 ml PBS 1X and 270  $\mu$ l of 37% formaldehyde to the plate and incubate on a shaking platform (gently shaking) for 10min at room temperature.
3. Stop crosslinking reaction by adding 1 ml **Glycine Buffer** to the plate. Incubate on a shaking platform (gently shaking) for 5min at room temperature.
4. Remove medium and wash cells twice using 10 ml ice cold PBS 1X to the plate.
5. Remove medium and add 5 ml ice cold PBS 1X. Scrape cells from the plate and collect them into a 15 ml or 50 ml conical tube.
6. Centrifuge at 800 x g at room temperature for 5 min to pellet cells.
7. Remove supernatant and resuspend cell pellet in 0.5 ml **Lysis Buffer I**.  
**Note : We recommend using 0.5ml of Lysis Buffer I and II for one plate containing  $2.5 \times 10^6$  cells.**
8. Centrifuge at 800 x g at room temperature for 6 min.
9. Remove supernatant and resuspend cell pellet in 0.5 ml **Lysis Buffer II**.
10. Centrifuge at 800 x g at room temperature for 6min.

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11. During centrifugation, prepare a Lysis Buffer III supplemented with Protease Inhibitors Cocktail.
12. Remove supernatant and resuspend cell pellet in 0.1 ml **Lysis Buffer III containing Protease Inhibitors Cocktail**.

### 2.3.2 Chromatin Shearing



Sonication is a critical step that must be optimized.

#### Advice on sonication

- Sonicate chromatin to an average length of about 500 bp is recommended.
- In order to monitor the sonication process, extract total DNA from sheared chromatin and analyse it on 1% agarose gel for each sonication cycle.
- In order to avoid any delay in gel migration, due to proteins fixed onto DNA, we strongly recommend to reverse sheared chromatin before agarose gel analysis.
- Power, time and number of sonication cycles have to be tuned depending on sonicator, cell type, crosslinking degree and protein of interest.
- To avoid excessive heating that denatures the DNA, the total sonication time is usually divided into cycles of sonication (~5 cycles each of 15s, with a pause point between cycles). Be sure to keep samples on ice to prevent chromatin from degradation.
- The chromatin can be stored at -80°C for months, depending on the targeted protein.

1. If desired, remove 5 µl of cell lysate for agarose analysis of unsheared chromatin.
2. Sonicate cell lysate on wet ice.
3. Centrifuge at 13.000 x g at room temperature for 10 min to remove insoluble material. Collect the supernatant.
4. If desired remove 5 µl aliquot for agarose gel analysis of the sheared chromatin.
5. 100 µl aliquot is recommended for one immunoprecipitation.



Freshly sheared chromatin is recommended for high quality ChIP.

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### 2.3.3 Immunoprecipitation of crosslinked protein / DNA

The follow protocol is done for the *automate-ChIP-Flash* procedure.

 Please follow the manual instruction of the instrument to optimize the automated step.



Microtiter Deep well 96 plate  
(Cat.# 21102)



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

#### 1. Prepare the plate

Please, find below default Chromatin IP protocols:

Automated ChIP protocol	Duration	IP DNA	Sample for Blot	Which protocol to start?	protocol
<b>ChIP Flash</b>	1h30	30min	No	ChIP Flash to perform chromatin sonication optimization	ChIPFlash v1.0
<b>ChIP Blot</b>	1h30	30min	Yes 200µl sample to perform Western Blotting	ChIP Blot to validate antibody quality	ChIPBlot v1.0
<b>ChIP O/N Step 1</b>	30min	O/N TA or 4°C (outside AutoMAg)	No	For less protein expression overnight immunoprecipitation	ChIPONstep1 v1.0
<b>ChIP O/N Step 2</b>	30min	No	No		ChIPONstep2 v1.0
<b>ChIP Flash washing steps plus</b>	1h40	30min	No	ChIP Flash with 6 possibilities of washing steps (2 DeepWell plates are used)	ChIPFlashWSP v1.0

- **ChIP Flash/ ChIP Blot:**

Add the following reagents to the rows. Note that row B is reserved for the tip comb and should be left empty.

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Plate name and type	Row	Row name	Content	Reagent/Sample volume per well
Microtiter Deep well 96 plate	A	Elution Step	Elution Buffer Proteinase K	200µl 10µl
	B	Tip	12-tip comb	Empty
	C	ChIP-Adembeads	ChIP-Adembeads	50µl
	D	Blocking Step	Blocking Buffer	250µl
	E	Binding Step	IP Buffer antibody	100µl xµl
	F	ChIP Step	IP Buffer Chromatin	900µl 100µl
	G	Washing Step I+II	Washing Buffer	500µl
	H	Washing Step III+IV	Washing Buffer	500µl

### Note:

- Regarding ChIP Blot protocol, the “Pause” step is carry out after the washing steps, please recover 200µl of sample to perform Western Blotting.

- **ChIP O/N**

Step 1:

Plate name and type	Row	Row name	Content	Reagent/Sample volume per well
Microtiter Deep well 96 plate	A	Empty	Empty	Empty
	B	Tip	12-tip comb	Empty
	C	ChIP-Adembeads	ChIP-Adembeads	50µl
	D	Blocking Step	Blocking Buffer	250µl
	E	Binding Step	IP Buffer antibody	100µl xµl
	F	Empty	Empty	Empty
	G	Empty	Empty	Empty
	H	Empty	Empty	Empty

## ChIP Adem-Kit – AutoMag Solution

Step 2:

Plate name and type	Row	Row name	Content	Reagent/Sample volume per well
<b>Microtiter Deep well 96 plate</b>	A	Elution Step	Elution Buffer	200µl
			Proteinase K	10µl
	B	Tip	12-tip comb	Empty
	C	Complex ChIP	Chromatin-Ab-Beads	1000µl max
	D	Washing Step I	Washing Buffer	500µl
	E	Washing Step II	Washing Buffer	500µl
	F	Washing Step III	Washing Buffer	500µl
	G	Washing Step IV	Washing Buffer	500µl
H	Washing Step V	Washing Buffer	500µl	

- **ChIP Flash washing steps plus:**

Deep well 96 plate 1:

Plate name and type	Row	Row name	Content	Reagent/Sample volume per well
<b>Microtiter Deep well 96 plate</b>	A	Elution Step	Elution Buffer	200µl
			Proteinase K	10µl
	B	Tip	12-tip comb	Empty
	C	ChIP-Adembeads	ChIP-Adembeads	50µl
	D	Blocking Step	Blocking Buffer	250µl
	E	Binding Step	IP Buffer	100µl
			antibody	xµl
	F	ChIP Step	IP Buffer	900µl
Chromatin			100µl	
G	Washing Step I	Washing Buffer	500µl	
H	Washing Step II	Washing Buffer	500µl	

## ChIP Adem-Kit – AutoMag Solution

Deep well 96 plate 2:

Plate name and type	Row	Row name	Content	Reagent/Sample volume per well
Microtiter Deep well 96 plate	A	Empty	Empty	Empty
	B	Empty	Empty	Empty
	C	Washing Step III	Washing Buffer	500µl
	D	Washing Step IV	Washing Buffer	500µl
	E	Washing Step V	Washing Buffer	500µl
	F	Washing Step VI	Washing Buffer	500µl
	G	Empty	Empty	Empty
	H	Empty	Empty	Empty

### Note:

- The amount of antibody added should be in excess and determined for each ChIP. Usually 1 to 3 µg of immunoprecipating antibody is recommended for each ChIP reaction.
- For mock IP, use the non-immune IgG fraction from the same species in which the specific antibodies were produced.

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



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

3. Load the plate and start the ChIP automated procedure.

Switch ON the Instrument and make sure that you are using the 12-pin magnet head and heating block. Start the ChIP automated protocol. Insert the plate into the instrument as indicated on the Instrument display and press **OK**.

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Automated ChIP protocol	Duration	IP DNA	Sample for Blot	Which protocol to start?	protocol
<b>ChIP Flash</b>	1h30	30min	No	ChIP Flash to perform chromatin sonication optimization	ChIPFlash v1.0
<b>ChIP Blot</b>	1h30	30min	Yes 200µl sample to perform Western Blotting	ChIP Blot to validate antibody quality	ChIPBlot v1.0
<b>ChIP O/N Step 1</b>	30min	O/N TA or 4°C (outside AutoMag)	No	For less protein expression overnight immunoprecipitation	ChIPONstep1 v1.0
<b>ChIP O/N Step 2</b>	30min	No	No		ChIPONstep2 v1.0
<b>ChIP Flash washing steps plus</b>	1h40	30min	No	ChIP Flash with 6 possibilities of washing steps (2 DeepWell plates are used)	ChIPFlashWSP v1.0

#### 4. After the run is completed, remove the plate and store immunoprecipitated DNA.

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

##### 2.3.4 Western Blotting control

- 1- If desired, remove 200 µl of sample for blot into a microtube.
- 2- Place the tube on the magnet (AdemMag MODULO #20105) until supernatant clearing. Discard the supernatant.
- 3- Resuspend the beads in 25 µl of Elution Buffer
- 4- Incubate 15 min at 65°C
- 5- Place the tube on the magnet for at least 1min or until supernatant clearing. Collect the supernatant and use for western blot.

##### 2.3.5 Crosslinking reversion and DNA purification

**Incubate overnight the Elution fraction at 65°C under mixing (300 rpm).**

Purify the DNA using ChIP DNA prep Adem-kit:

**ChIP DNA Prep Adem-kit manual and automation version (# 06240)  
ChIP DNA Prep Adem-kit AutoMag Solution Prefilled Reagents (# 06241)**

- **Binding step:**

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.

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- **Washing steps:**

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. Magnetize the particle suspension at least 5 minutes, and discard carefully the supernatant without disturbing the pellet of magnetic particle.
2. Remove the microtube from the magnet and resuspend the pellet of magnetic particles in **500µL of ChIP DNA Washing Buffer**

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3. Magnetize the particle suspension at least 5 minutes, and discard carefully the supernatant without disturbing the pellet of magnetic particle.
4. Remove the microtube from the magnet and resuspend the pellet of magnetic particles in **250µL of ChIP DNA Washing Buffer**

- **Drying step:**

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.

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**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

- **Elution step:**

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.

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**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 with Traditional PCR, Real-Time PCR, Digital PCR and Sequencing applications.

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### 2.3.6 PCR Controls

**Note:**

- **Optimize your PCR protocol: determine the optimal number of PCR cycles (no or low PCR product with the mock/ strong PCR product with the specific antibody).**
- **Starting with 30 cycles is recommended.**

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### **PCR negative controls:**

- PCR with DNA from sample immunoprecipitated with non-immune IgG fraction from the same species of the antibodies.
- PCR using DNA from ChIP samples, and specific primers for a DNA region where your antigen of interest is not bound.

**PCR positive controls:** PCR using input DNA

## Troubleshooting

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

LOW OR NO PCR PRODUCTS		
STEP	TROUBLES	SUGGESTION
<b>CROSSLINKING</b>	Not enough or too much crosslinking	Efficient fixation of protein to chromatin is a crucial step. It is important to optimize the fixation step by testing different formaldehyde concentrations, different incubation times. Use high quality formaldehyde.
<b>CELL LYSIS</b>	Insufficient cell number	Increase cell number.
	Insufficient cell lysis	Do not use too many cells per amount of Lysis Buffer. Follow the protocol step 2.3.1.
	Protein degradation during lysis can occur	Perform cell lysis at 4°C or on ice. Keep samples and Buffers on ice. Add Protease Inhibitors to the Lysis Buffer III.

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<b>CHROMATIN SHEARING</b>	Not enough or too much chromatin	Optimal shearing conditions are important for ChIP efficiency and must be optimized for each cell type, fixation protocol and sonicator apparatus. Follow advices on sonication to obtain the appropriate sized DNA. To analyze the shearing, reverse crosslink, purify DNA and do a 1% agarose gel.
	Denaturation of proteins during sonication	Keep the sample on ice during sonication.
<b>IMMUNOPRECIPITATION</b>	Antibody doesn't recognize protein in fixed chromatin	Choose ChIP or IP grade antibody directed to a different epitope of the protein. Decrease time of formaldehyde fixation or its concentration.
	Incorrect antibody class of isotype	Check if the subclass and the isotype of the antibody match well with pA /pG affinity.
	High background	Perform a preclearing step. -Incubate each chromatin with 25 µl blocked ChIP-Adembeads for 1 h at room temperature. -Place the tube on the magnet for at least 1 min or until supernatant clearing. -Collect the supernatant and use it in the step 9 / chapter 2.3.3.
	Beads agglutination	Beads agglutination may occur after immunoprecipitation step. This phenomenon corresponds to the capture of the antibody by the beads. During washing steps, resuspend thoroughly the beads by pipeting to avoid non specific binding.
<b>REVERSION</b>	Incorrect temperature, insufficient time for DNA release and reversion	Follow the protocol steps 2.3.5. Check if magnetic beads have been eliminated before incubation at 65°C.
<b>PCR</b>	Incorrect PCR conditions	Check if all PCR components are added. Ensure the designed primers are specific to the target sequence. Increase the number of cycles for PCR reaction. Increase or reduce amount of DNA added.

## 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
<b>04643</b>	ChIP Adem-Kit for AutoMag Solution	1 x 24
<b>06241</b>	ChIP DNA Prep Adem-Kit AutoMag Solution	48

- **Instrument and consumables**

CAT NO.	PRODUCT	PACKAGE SIZE
<b>21101</b>	Instrument for AutoMag Solution	Each
<b>21102</b>	Microtiter Deepwell 96 plate	50 pcs
<b>21103</b>	12-tip comb for 96 Deepwell plate	50 pcs
<b>21105</b>	Combi pack for 96 Deepwell plate	Each