

# Nuclear Complex Co-IP Kit

(version B2)

Catalog No. 54001

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## Introduction

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Co-Immunoprecipitation (Co-IP) is a powerful method used to study protein/protein interactions. In Co-IP, one antibody is used to immunoprecipitate a target antigen and also co-precipitate any bound interacting proteins within a sample. These protein complexes are then captured by antibody binding beads (such as Protein A or G). This is followed by elution of the antibody-bound proteins, SDS-PAGE and Western blot using a second antibody targeted against one of the bound, interacting proteins. Traditional methods for performing Co-IP are not optimal for studying DNA-binding protein complexes as the complexes are often disrupted during the extraction process. In addition, many unstable protein complexes can be affected by the salt and detergent composition of the buffers used in the immunoprecipitation process.

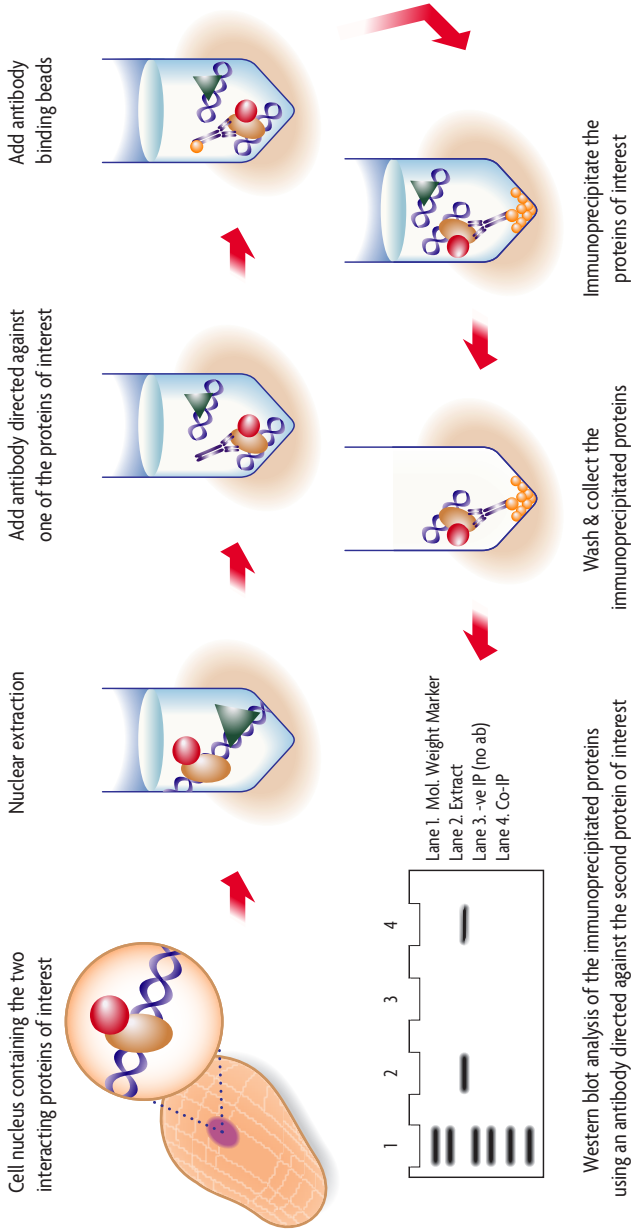
Because of this, Active Motif's Nuclear Complex Co-IP Kit has been developed specifically for the preparation and immunoprecipitation of nuclear protein complexes from cell or tissue samples. The kit is composed of two optimized modules: nuclear extract preparation and Co-IP. The kit's extraction module provides a simple, fast and effective method to obtain and maintain protein complexes contained in nuclear compartments of the cell, specifically those previously bound to DNA. The kit's versatile Co-IP module enables the user to study many different protein/protein interactions of varying strength.

In the kit's extraction process the cells are collected in ice-cold PBS in the presence of Phosphatase Inhibitors, which limits further protein modifications (expression, proteolysis, dephosphorylation, etc.). The cells are then resuspended in Hypotonic Buffer to swell the cell membrane and make it fragile. Addition of Detergent causes leakage of the cytoplasmic proteins into the supernatant. After removal of the cytoplasmic fraction, the nuclei are lysed and the nuclear proteins are recovered in a low-salt buffer in the presence of the Protease Inhibitor Cocktail and PMSF. This is followed by the addition of a proprietary Enzymatic Shearing Cocktail. The use of low-salt buffers protects protein complexes in the nucleus; DNA digestion allows a gentle release of undissociated protein complexes from the DNA. After the protein complexes are collected, an immunoprecipitation reaction is carried out to detect the bound proteins. In the immunoprecipitation module, two different immunoprecipitation buffers with either a low or high stringency starting composition are provided. In addition, detergent and salt are provided separately to enable the user to vary the salt and detergent concentrations. The addition of salt and detergent is ideal for use with robust protein/protein interactions because such conditions reduce background. However, as unstable protein complexes may not withstand high stringencies, the kit format enables stringency to be modified as required by each complex.

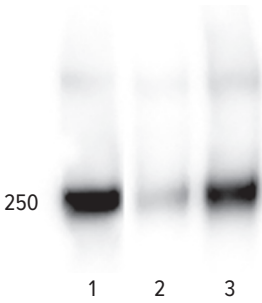
The Nuclear Complex Co-IP Kit provides sufficient reagents to perform 50 Co-IP experiments. It includes optimized nuclear protein complex extraction reagents, immunoprecipitation buffers and a comprehensive protocol to provide a simplified method to capture and detect protein complexes.

<b>product</b>	<b>format</b>	<b>catalog no.</b>
Nuclear Complex Co-IP Kit	50 rxns	54001
Protein G Agarose Columns	30 rxns	53039

# Flow Chart of Process



## Kit Performance



**Figure 1: Co-IP of the p33 subunit of the RNA pol II complex.**

HeLa cells were grown to confluence on 100 mm plates and nuclear extracts were prepared using the kit's extraction reagents. For IP experiments, the stringency of the IP High Buffer was increased by supplementing with NaCl (150 mM Final) and Detergent (1% Final). 100 µg of nuclear extract was used per IP reaction and incubated with either 2 µg p33 antibody or no antibody (negative control). Protein G beads were added to each IP reaction. Following the IP, 2X sample buffer was added to each IP reaction, samples were boiled and run on an SDS-PAGE gel. Western blot analysis was performed using RNA pol II mouse mAb at 0.1 µg/ml followed by anti-mouse HRP at 1:1000. Detection of the p33/RNA pol II complex by the RNA pol II antibody (lane 3) demonstrates that the Co-IP was successful in maintaining the protein complex. The input HeLa extract (lane 1) was run as a control for the western blot using 0.1 µg/ml RNA pol II.

Lane 1: Western blot control – 10 µg HeLa extract  
Lane 2: Negative Control – no antibody added in IP  
Lane 3: Co-IP: IP p33/WB RNA pol II

## Kit Components and Storage

Kit components can be stored at -20°C prior to first use. Then, we recommend storing each component at the temperatures recommended in the table below:

Reagents	Quantity	Storage / Stability
10X Hypotonic Buffer	50 ml	4°C for 6 months
Phosphatase Inhibitors	40 ml	4°C for 6 months
Protease Inhibitor Cocktail	626 µl	-20°C for 6 months
10X PBS	80 ml	4°C for 6 months
Detergent	22.8 ml	RT for 6 months
100 mM PMSF	50 µl	-20°C for 6 months
0.5 M EDTA	200 µl	4°C for 6 months
Digestion Buffer	10 ml	4°C for 6 months
Enzymatic Shearing Cocktail	50 µl	-20°C for 6 months
5X IP Low Buffer	22.5 ml	4°C for 6 months
5X IP High Buffer	22.5 ml	4°C for 6 months
Dithiothreitol (DTT)	225 µl	-20°C for 6 months
5 M NaCl	6.8 ml	RT for 6 months
BSA	300 mg	4°C for 6 months

## Additional materials required

- 5 and 10 ml pipettes
- Pipettors
- Cell scraper
- 15 ml conical tubes
- Microcentrifuge tubes
- Centrifuge (with swinging buckets adapted to 15 ml conical tubes) and microcentrifuge pre-cooled to 4°C
- Rocking platform
- Distilled water
- Apparatus to rotate tubes end-to-end at 4°C (for co-immunoprecipitation)
- Protein G Agarose Columns (Active Motif Catalog No. 53039) or Antibody Binding Beads (such as Protein A or G beads)
- Antibodies for IP and Western blot
- 37°C Water Bath
- Reagents and equipment for performing SDS-PAGE and Western blot

## Protocols

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### Nuclear Extraction – Buffer Preparation and Recommendations

#### Preparation of PBS/Phosphatase Inhibitors

The Phosphatase Inhibitors solution should be a clear yellow color. It may precipitate during storage and become turbid in appearance. If this occurs, heat at 50°C for 10 minutes. To make nuclear extract from a 100 mm plate of cells, prepare 8 ml of PBS/Phosphatase Inhibitors solution as follows: mix 0.8 ml 10X PBS in 6.8 ml distilled water, then add 0.4 ml Phosphatase Inhibitors. The Phosphatase Inhibitors lose their activity 24 hours after dilution. Therefore, use the PBS/Phosphatase Inhibitors solution the same day it is prepared and discard any remaining solution.

#### Preparation of 1X Hypotonic Buffer

To make nuclear extract from a 100 mm plate of cells, prepare 500 µl of 1X Hypotonic Buffer as follows: mix 50 µl 10X Hypotonic Buffer and 450 µl distilled water. Any remaining 1X Hypotonic Buffer can be stored at 4°C for 1 week.

#### Preparation of Complete Digestion Buffer

To make nuclear extract from a 100 mm plate of cells, prepare 100 µl of Complete Digestion Buffer as follows: mix 98.5 µl Digestion Buffer with 1 µl Protease Inhibitor Cocktail (PIC) and 0.5 µl PMSF. The PMSF and PIC lose their activity 24 hours after dilution. Therefore, use the Complete Digestion Buffer solution the same day that it is prepared and discard any remaining solution.

## Quick Chart for Preparing Buffers

Reagents to Prepare	Components	60 mm plate or 3.2 x 10 <sup>6</sup> cells	100 mm plate or 8.8 x 10 <sup>6</sup> cells	150 mm plate or or 2 x 10 <sup>7</sup> cells
PBS/Phosphatase Inhibitors	10X PBS	0.4 ml	0.8 ml	1.6 ml
	Distilled water	3.4 ml	6.8 ml	13.6 ml
	Phosphatase Inhibitors	0.2 ml	0.4 ml	0.8 ml
	<b>TOTAL REQUIRED</b>	<b>4.0 ml</b>	<b>8.0 ml</b>	<b>16.0 ml</b>
1X Hypotonic Buffer	10X Hypotonic Buffer	25.0 µl	50.0 µl	100.0 µl
	Distilled water	225.0 µl	450.0 µl	0.9 ml
	<b>TOTAL REQUIRED</b>	<b>250.0 µl</b>	<b>500.0 µl</b>	<b>1.0 ml</b>
Complete Digestion Buffer	100 mM PMSF	0.25 µl	0.5 µl	1.0 µl
	Digestion Buffer	49.25 µl	98.5 µl	197.0 µl
	Protease Inhibitor Cocktail	0.50 µl	1.0 µl	2.0 µl
	<b>TOTAL REQUIRED</b>	<b>50.0 µl</b>	<b>100.0 µl</b>	<b>200.0 µl</b>

## Preparation of Nuclear Extract

### Starting from Cells:

The following protocol is based on samples of approximately  $8.8 \times 10^6$  cells, which correspond to HeLa cells grown to confluence in a 100 mm tissue culture plate, and will yield approximately 0.20-0.35 mg of protein. Each sample is one extraction reaction. Prepare PBS/Phosphatase Inhibitors, Hypotonic Buffer and Complete Digestion Buffer as described above in the section Buffer Preparation. Adjust the volumes accordingly using the chart above if using plates of different sizes. Place buffers and any tubes needed on ice before beginning assay.

### Section A: Cell Collection

1. Aspirate media out of dish. Wash with 5 ml ice-cold PBS/Phosphatase Inhibitors. Aspirate solution out then add 3 ml ice-cold PBS/Phosphatase Inhibitors.
2. Remove cells from dish by gently scraping with cell lifter. Transfer cells to a pre-chilled 15 ml conical tube.
3. Centrifuge cell suspension for 5 minutes at 430 x g in a centrifuge pre-cooled at 4°C.
4. Discard supernatant. Keep cell pellet on ice. At this point the protocol can be continued or the pellet can be frozen at -80°C.

### Section B: Isolation of the Nuclei

1. Gently resuspend cell pellet in 500 µl 1X Hypotonic Buffer by pipetting up and down several times. Transfer to a pre-chilled microcentrifuge tube. Incubate for 15 minutes on ice.
2. Add 25 µl Detergent and gently pipet up and down 3-5 times to mix.
3. Centrifuge suspension for 30 seconds at 14,000 x g in a microcentrifuge pre-cooled at 4°C.



4. Discard supernatant (cytoplasmic fraction). Keep pellet (nuclear fraction) on ice.

### Section C: Nuclear Fraction Digestion and Collection

1. Resuspend nuclear pellet in 100  $\mu$ l Complete Digestion Buffer by pipetting up and down 3-5 times.
2. Add 0.5  $\mu$ l of the Enzymatic Shearing Cocktail. Vortex gently for 2 seconds.
3. Incubate suspension for 10 minutes at 37°C.

**Note:** If you are studying protein/protein interactions that may be fragile or unstable, perform this Enzymatic Shearing Cocktail incubation at 4°C for 90 minutes.

4. Add 2  $\mu$ l 0.5 M EDTA to stop the reaction. Vortex gently on a low setting for 2 seconds and place on ice for 5 minutes.
5. Centrifuge for 10 minutes at 14,000  $\times g$  in a microcentrifuge pre-cooled at 4°C. Transfer supernatant into a pre-chilled microcentrifuge tube.
6. Save a small aliquot for protein quantification by a Bradford-based assay. Aliquot and store at -80°C. Avoid freeze/thaw cycles.

**Note:** The presence of certain detergents may interfere with protein determination assays, such as Bradford or BCA. We therefore recommend using the Complete Digestion Buffer as the blank and performing a 1:50 or 1:250 dilution of your samples. Alternatively, try Active Motif's ProStain™ Protein Quantification Kit, which is ideal for quantifying extracts because it offers greater sensitivity and resistance to many contaminating reagents used in extract preparation.

### Starting from Tissue:

#### Section D: Tissue Homogenization

1. Weigh tissue and dice into very small pieces using a clean razor blade. Collect pieces in a pre-chilled, clean Dounce homogenizer.
2. On ice, add 3 ml ice-cold 1X Hypotonic Buffer supplemented with DTT and Detergent (3  $\mu$ l of the provided 1 M DTT and 3  $\mu$ l of the provided Detergent) per gram of tissue and homogenize. Incubate on ice for 15 minutes.
3. Centrifuge for 10 minutes at 850  $\times g$  at 4°C.
4. At this point, the tissue is homogenized. However, most of the cells are not yet lysed. Therefore, continue the procedure with the cell pellet at Section B, No. 1 of the Nuclear Extract protocol for cells (page 5), based on a 150 mm plate ( $2 \times 10^7$  cells).

## Co-IP – Buffer Preparation and Recommendations

The Nuclear Complex Co-IP Kit has been designed to enable the study of a variety of protein/protein interactions. The Kit includes two IP Buffers with different stringencies: IP High has a higher stringency than IP Low. Each IP Buffer can be further supplemented with the provided salt and detergent depending on the complex being studied. Increased stringency is suitable for stable protein complexes as it reduces background levels without disrupting protein interactions. IP Buffers containing low salt and detergent enable the detection of more weakly bound protein complexes. If the strength of the protein complex is not known, we recommend performing a series of experiments to determine the optimal buffer stringency as outlined in the table below.

Approach	IP Buffer	5 M NaCl	Detergent	5 M NaCl for Wash Buffer
1. No supplements	1X High	N/A	N/A	N/A
	1X Low	N/A	N/A	N/A
2. Addition of salt	1X High	150 mM Final	N/A	N/A
	1X Low	150 mM Final	N/A	N/A
3. Addition of salt and detergent	1X High	150 mM Final	1% Final	N/A
	1X Low	150 mM Final	1% Final	N/A
4. Addition of salt and detergent, and extra salt for final Wash Step	1X High	150 mM Final	1% Final	300 mM Final
	1X Low	150 mM Final	1% Final	300 mM Final

### Preparation of IP Buffer

The IP Buffers should be supplemented with fresh Protease Inhibitor Cocktail (PIC) and DTT before use. However, if DTT is not compatible with the protein complex being studied, do not use.

**Note:** IP Incubation Buffer should be prepared fresh on Day 1. IP Wash Buffer should be prepared fresh on Day 2. If you wish to increase stringency of the IP Buffers add NaCl and Detergent as listed below. If you do not wish to increase stringency simply add dH<sub>2</sub>O to recommended Total Volume.

	Volume Required for 1 Co-IP		Volume Required for 5 Co-IP's	
	Day 1	Day 2	Day 1	Day 2
	IP Incubation	IP Wash	IP Incubation	IP Wash
5X IP Buffer	100 µl	800 µl	500 µl	4000 µl
PIC	2.5 µl	8 µl	12.5 µl	40 µl
1 M DTT	0.5 µl	4 µl	2.5 µl	20 µl
5 M NaCl*	7.5 µl	60 µl	37.5 µl	300 µl
Detergent*	45 µl	360 µl	225 µl	1800 µl
dH <sub>2</sub> O	X µl	X µl	X µl	X µl
<b>Total Volume</b>	<b>500 µl</b>	<b>4000 µl</b>	<b>2500 µl</b>	<b>20 ml</b>

\* Add these components to the IP Incubation and Wash Buffers only if you wish to increase stringency.

# Co-Immunoprecipitation Protocol

**IMPORTANT:** Please read the previous section, Co-IP – Buffer Preparation and Recommendations before starting the assay. When possible, all IP steps should be performed on ice. The protocol below provides volumes sufficient for one IP experiment and can be scaled up as needed. We also recommend including one IP negative control (no antibody added in the IP reaction).

## DAY 1: Preparation of Antibody/Extract mixture

1. Prepare IP Incubation Buffer as described in the above section. Each IP reaction will require 500  $\mu$ l.
2. For preparation of samples, we recommend using 100-500  $\mu$ g of extract and 1-5  $\mu$ g of antibody diluted to 500  $\mu$ l in IP Incubation Buffer in a 1.5 ml microcentrifuge tube. Keep tubes on ice.
3. Incubate each Antibody/Extract mixture overnight at 4°C on a rotator.

## DAY 2: Addition of Antibody binding beads and IP of the Proteins of Interest

Active Motif recommends the use of its Protein G Agarose Columns (Catalog No. 53039) for the capture of the IP reaction. The Protein G agarose beads supplied in the column are pre-washed and ready to use. If using an alternative source of Protein A or G beads, the beads should be thoroughly washed before use. Follow the protocol for the antibody capture method being used.

1. Prepare IP Wash Buffer as described in the above section. Each IP reaction will require 4 ml of the IP Wash Buffer. Keep the buffer on ice until ready to use.
2. Prepare a 100 mg/ml BSA solution by dissolving the provided BSA in 2.8 ml dH<sub>2</sub>O and vortex to mix. Store unused BSA at -20°C.
3. For each IP reaction, transfer 1.5 ml of IP Wash Buffer to a new tube. Supplement with 1 mg/ml BSA by adding 15  $\mu$ l BSA to 1.5 ml IP Wash Buffer. Keep the buffer on ice until ready to use.

## If using Protein G Column-based Capture

1. Add the IP reaction directly to the column. Cap the column tightly to ensure it is sealed. If desired, use parafilm to seal the cap. (Do not remove the tab from the column.)
2. Incubate antibody/bead complex for 1 hour at 4°C on a rotator.
3. During the incubation, prepare a 2X Reducing Buffer (130 mM Tris pH 6.8, 4% SDS, 0.02% Bromophenol blue, 100 mM DTT).
4. Following the incubation, spin the column at 1250 x g for 1 minute to collect liquid from inside of the cap.
5. The columns are designed to fit within an empty 1 ml pipet tip box which will serve as a stand for wash steps (see Appendix A for set up). Remove the cap and the tab from the bottom of the column and place the columns in the empty tip box. Add 500  $\mu$ l ice-cold IP Wash Buffer supplemented with BSA to the column. Allow flow-through to occur by gravity.
6. Repeat the wash step twice with 500  $\mu$ l ice-cold IP Wash Buffer supplemented with BSA.

7. Add 500  $\mu$ l ice-cold IP Wash Buffer (no BSA) to each column.
 

**Note:** To increase the stringency of this wash step, add 22.5  $\mu$ l 5 M NaCl to each 500  $\mu$ l of IP Wash buffer to be used.
8. Repeat the wash step two more times with 500  $\mu$ l ice-cold IP Wash Buffer (no BSA).
9. Transfer the column to a microcentrifuge tube and centrifuge at 1250 x g at 4°C for 3 minutes to remove residual IP Wash Buffer.
10. Transfer the column to a new microcentrifuge tube.
11. Add 25  $\mu$ l of 2X Reducing Buffer to each column and let stand for 1 minute at room temperature. Centrifuge at 1250 x g at room temperature for 3 minutes to elute the IP reaction.
12. Add 5  $\mu$ l of pure glycerol to each 25  $\mu$ l elution. Pipet up and down to mix.
13. Boil samples at 95-100°C for 5 minutes. Allow sample to cool to room temperature and quick spin to collect condensation. Load gel for electrophoresis and subsequent Western blotting.

#### If using Protein A or G Bead Capture in Solution

1. Antibody binding beads (Protein A or G) must be washed thoroughly before addition to the Antibody/Extract mixture from Day 1. For each IP reaction, aliquot 50  $\mu$ l of resuspended beads to fresh 1.5 ml microcentrifuge tubes.
2. Add 250  $\mu$ l IP Wash Buffer to each tube and flick to mix. Centrifuge at 1430 x g for 30 seconds at 4°C. Remove supernatant. Repeat wash step twice and place beads on ice.
3. Add 40  $\mu$ l ice-cold IP Wash Buffer to the pelleted beads (total volume will exceed 60  $\mu$ l).
4. Add 60  $\mu$ l of the washed beads to each Antibody/Extract tube from Day 1.
5. Incubate antibody/bead complex for 1 hour at 4°C on a rotator.
6. During the incubation prepare a 2X Reducing Loading Buffer (130 mM Tris pH 6.8, 4% SDS, 0.02% Bromophenol blue, 100 mM DTT, 20% glycerol)
7. Following the incubation, centrifuge tubes at 1430 x g for 30 seconds at 4°C. Remove and discard the supernatant.
8. Add 500  $\mu$ l ice-cold IP Wash Buffer supplemented with BSA to each tube. Centrifuge at 1430 x g for 30 seconds at 4°C. Remove and discard the supernatant. Repeat wash step twice with 500  $\mu$ l ice-cold IP Wash Buffer supplemented with BSA. Do not vortex or flick tubes to mix. Keep tubes on ice when possible.
9. Add 500  $\mu$ l ice-cold IP Wash Buffer (no BSA) to the pelleted beads. Centrifuge at 1430 x g for 30 seconds at 4°C. Remove supernatant. Repeat wash step twice with 500  $\mu$ l ice-cold IP Wash Buffer (no BSA). Do not vortex or flick tubes to mix. Keep tubes on ice when possible.
 

**Note:** To increase the stringency of this wash step, add 22.5  $\mu$ l 5M NaCl to each 500  $\mu$ l of IP Wash Buffer to be used.
10. Resuspend each bead pellet in 8  $\mu$ l of 2X Reducing Loading Buffer and boil samples at 95-100°C for 5 minutes.

11. Allow sample to cool to room temperature, spin briefly to collect condensation and apply to gel for electrophoresis and subsequent Western blotting.

## Western Blotting Protocol

For your convenience, a Western blotting protocol is provided below. When performing Western blot, individual optimization of antigen and antibody quantities may be required in order to detect the protein of interest.

### Electrophoresis

1. Load sample onto an appropriate SDS-PAGE gel and run until migration front reaches bottom of gel.

### Transfer

2. Transfer protein to polyvinylidene difluoride (PVDF) membranes at 400 mA for 90 minutes using Transfer Buffer (25 mM Tris-HCl, pH 8.3, 192 mM glycine, 15% methanol).

### Blocking

3. Block membranes by incubating overnight at 4°C with shaking in 5% Blotto (5% dry, non-fat skim milk powder in PBS (pH 7.4)). The addition of 0.1% Tween 20 to all Blotto and PBS solutions may increase the antibody specificity and decrease the background.

### Incubation with Primary Antibody

4. Rinse membranes with PBS (pH 7.4). Dilute the antibody (at the appropriate dilution) in 5% Blotto. Incubate for 1 hour at 37°C, 2 hours at RT (room temperature) or overnight at 4°C with agitation.
5. Decant antibody solution. Wash the membrane 5 times for 5-10 minutes at RT in PBS. Washing should be performed with vigorous agitation over a minimum 30-minute period.

### Incubation with Secondary Antibody

6. Incubate membranes with secondary HRP conjugate diluted in 5% Blotto for 1 hour at RT with gentle shaking. The dilution of the secondary antibody conjugate will vary according to manufacturer's specifications.
7. Repeat step 5.
8. Wash membrane with PBS for 5 minutes with agitation before proceeding to the chemiluminescence reaction.

### Chemiluminescence Reaction

9. Prepare and use the chemiluminescent substrate according to the manufacturer's instructions.

# Appendix

## Section A. Protein G Agarose Columns Wash Stand

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The Protein G Agarose columns are designed to fit within an empty 1 ml pipet tip box which will serve as a stand for wash steps. Remove the cap and the tab from the bottom of the column and place the columns in the empty tip box. All wash steps can be performed using gravity flow.



## Section B. Section A. Troubleshooting Guide

Problem/question	Possible Cause	Recommendation
No or low signal of IP'd proteins by Western blot	Proteins of interest are not located in the nucleus or their level of expression is very low.	Confirm presence of proteins of interest in the nucleus and nuclear extract. Alternatively, use more extract in the IP reaction (up to 500 µg per IP).
	Target proteins lost or destroyed during sample preparation.	Confirm presence of proteins of interest in the nuclear extract.
	The protein/protein interaction of interest is dependent on specific induction conditions.	Optimize conditions of culture and induction.
	The protein/protein interaction of interest is not stable and dissociates during the extraction process.	Perform the Enzymatic Shearing Cocktail incubation for 90 minutes at 4°C instead of 10 minutes at 37°C.
	Amount of antibody used in the assay is too low.	Use more antibody in IP and/or WB; perform a titration to determine how much.
	Antibodies used in IP and/or WB are not suitable for these applications.	It is important to perform IP and WB controls, as antibodies raised against denatured antigen and native epitopes may not be recognized. IP Positive Control - IP antigen of interest and WB same antigen. WB Positive Control - direct WB analysis of the extract.
	The IP buffer is too stringent.	Use the IP High and IP Low buffers without the addition of salt and detergent. Favor use of IP Low buffer, which is less stringent.
	Large number of competing proteins present in the sample.	Centrifuge the extract at 20,000 x g for 20 minutes before adding antibody. This should remove insoluble aggregates.
	Interfering substances present in the nuclear extract.	Use the appropriate amount of inhibitors when performing the assay.
	Antibody breakdown.	Reduce concentration of DTT, or avoid use.
	Interference with the establishment or maintenance of the antigen-antibody complex.	Reduce concentration of detergent.
High background signal	Too much antibody used in IP and/or WB.	Titrate antibody concentrations and decrease primary antibody dilution or incubation time.
	Non-specific binding of other proteins to the Protein A/G beads or antibodies.	Pre-clear extracts with Protein A/G beads prior to addition of the primary antibody.
	Wash steps are not sufficiently stringent.	0.5% sodium deoxycholate can be added to the last 3 IP washes and may reduce signal from the negative (no antibody) control.
	Aggregated proteins in extracts.	Spin extracts at 20,000 x g for 20 minutes and discard pellet prior to addition of antibody.
	IgG heavy chains appear at 55 kDa, light chains appear at 28 kDa.	Use different antibody origins for IP and WB.

Problem/question	Possible Cause	Recommendation
Can whole-cell extracts be used?		If you wish to work with whole-cell extracts, we recommend use of Active Motif's Universal Magnetic Co-IP Kit, which contains reagents for making both nuclear or whole-cell extracts. It also utilizes Protein G Magnetic Beads and a magnet for the IP and Wash steps, which greatly reduces background.



## Technical Services

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If you need assistance at any time, please call or send an e-mail to Active Motif Technical Service at one of the locations listed below.

### Active Motif North America

Toll free: 877.222.9543  
Direct: 760.431.1263  
Fax: 760.431.1351  
E-mail: [tech\\_service@activemotif.com](mailto:tech_service@activemotif.com)

### Active Motif Europe

UK Free Phone: 0800/169 31 47  
France Free Phone: 0800/90 99 79  
Germany Free Phone: 0800/181 99 10  
Direct: +32 (0)2 653 0001  
Fax: +32 (0)2 653 0050  
E-mail: [eurotech@activemotif.com](mailto:eurotech@activemotif.com)

### Active Motif Japan

Direct: +81 (0)3 5225 3638  
Fax: +81 (0)3 5261 8733  
E-mail: [japantech@activemotif.com](mailto:japantech@activemotif.com)

### Active Motif China

Direct: (86)-21-20926090  
Cell Phone: 18521362870  
E-mail: [techchina@activemotif.com](mailto:techchina@activemotif.com)

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