



## Cross-linking Chromatin Immunoprecipitation (X-ChIP) Protocol

### 1. Cross-linking and Cell Harvesting

- 1.1. Start with two confluent 150cm<sup>2</sup> dishes (1x10<sup>7</sup>- 5x10<sup>7</sup> cells per dish). Cross-link proteins to DNA by adding formaldehyde drop-wise directly to the media to a final concentration of 0.75% and rotate gently at room temperature (RT) for 10min\*.
- 1.2. Add glycine to a final concentration of 125mM to the media and incubate with shaking for 5min at RT.
- 1.3. Rinse cells 2 x with 10ml cold PBS.
- 1.4. Scrape cells into 5ml cold PBS and transfer into 50ml tube.
- 1.5. Add 3ml PBS to dishes and transfer the remainder of the cells to the 50ml tube.
- 1.6. Centrifuge for 5min, 1,000g.
- 1.7. Carefully aspirate off supernatant and resuspend pellet in FA Lysis Buffer (750µl per 1x10<sup>7</sup> cells).

\* When using suspension cells, start with 1x10<sup>7</sup>- 5x10<sup>7</sup> cells and treat with both 0.75% formaldehyde and glycine as described above. Pellet cells by centrifugation (5mins, 1,000 g). Wash 3 x with cold PBS and resuspend pellet in FA Lysis Buffer (750µl per 1x10<sup>7</sup> cells). Proceed to Step 2.1.

### 2. Sonication

- 2.1. Sonicate lysate to shear DNA to an average fragment size of 500 to 1000bp. This will need optimizing as different cell lines require different sonication times. Analyze the fragment size on a 1.5% agarose gel.
- 2.2. After sonication pellet cell debris by centrifugation 30secs, 4°C, 8,000g. Transfer supernatant to a new tube\*\*. This chromatin preparation will be used for the immunoprecipitation (IP) in Step 4.
- 2.3. Remove 50µl of each sonicated sample, this sample is the INPUT. This is used to quantify the DNA concentration (see Step 3) and as a control in the PCR.

\*\* The lysed cells can be snap frozen in liquid nitrogen and stored at -70°C for up to 2 months. Avoid multiple freeze-thawing.

### 3. Determination of DNA concentration

- 3.1. The INPUT samples are used to calculate the DNA concentration for subsequent IPs. The DNA is purified using either a PCR purification kit (add 70µl of Elution Buffer and proceed to Step 3.2<sup>a</sup>) or phenol:chloroform (add 350µl of Elution Buffer and proceed to Step 3.2<sup>b</sup>).
- 3.2<sup>a</sup>. Add 2µl RNase A (0.5mg/ml). Heat with shaking at 65°C for 4-5hrs (or overnight) to reverse the crosslinks\*\*\*. DNA is purified using a PCR purification kit according to the manufacturer's instructions.
- 3.2<sup>b</sup>. Add 5ul proteinase K (20mg/ml). Heat with shaking at 65°C for 4-5hrs (or overnight) to reverse the crosslinks\*\*\*. The DNA is

phenol:chloroform extracted and ethanol precipitated in the presence of 10µl glycogen (5mg/ml). Resuspend in 100µl H<sub>2</sub>O.

- 3.3. To determine the DNA concentration, transfer 5µl of the purified DNA into a tube containing 995µl TE to give a 200-fold dilution and read the OD<sub>260</sub>. The concentration of DNA in µg/ml is OD<sub>260</sub> x 10,000. This is used to calculate the DNA concentration of the chromatin preparation.

\*\*\* The samples can be frozen and stored at -20°C after these steps.

### 4. Immunoprecipitation

- 4.1. Use the chromatin preparation from Step 2.2, an equivalent amount of approximately 25µg of DNA per IP is recommended. Dilute each sample 1:10 with RIPA Buffer. You will need one sample for the specific antibody and one sample for the beads only control.
- 4.2. Add primary antibody to all samples except the beads only control. The amount of antibody to be added should be determined empirically, 1-10µg of antibody per 25µg of DNA often works well.
- 4.3. Add 20µl of protein A/G beads (pre-adsorbed with sonicated single stranded herring sperm DNA and BSA\*\*\*\*) to all samples and IP overnight with rotation at 4°C.
- 4.4. Centrifuge the protein A/G beads for 1min, 2,000g and remove the supernatant.
- 4.5. Wash beads 3 x with 1ml Wash Buffer (centrifuge as above).
- 4.6. Wash beads 1 x with 1ml Final Wash Buffer (centrifuge as above).

\*\*\*\* Preparation of protein A/G beads with single stranded herring sperm DNA: Mix an equal volume of Protein A and Protein G beads and wash 3 x in RIPA Buffer. Aspirate RIPA Buffer and add single stranded herring sperm DNA to 75ng/µl beads and BSA to a final concentration of 0.1µg/µl beads. Add RIPA Buffer to twice the bead volume and incubate for 30min with rotation at RT. Wash once with RIPA Buffer and add RIPA Buffer to twice the bead volume.

### 5. Elution and reverse cross-links

- 5.1. Elute DNA by adding 120µl of Elution Buffer to the protein A/G beads and rotate for 15min, 30°C.
- 5.2. Centrifuge for 1min, 2,000g and transfer the supernatant into a fresh tube\*\*\*.
- 5.3. The DNA can be purified using a PCR purification kit (proceed with Step 3.2<sup>a</sup>) or phenol:chloroform (add 280µl of Elution Buffer and proceed with Step 3.2<sup>b</sup>).
- 5.4. DNA levels are quantitatively measured by real-time PCR. Primers and probes are often designed using software provided with the real-time PCR apparatus or the following website can be used.

[http://biotools.umassmed.edu/bioapps/primer3\\_www.cgi](http://biotools.umassmed.edu/bioapps/primer3_www.cgi)

## Solutions

FA Lysis Buffer	50ml	Stock Solution
50 mM HEPES-KOH pH7.5	2.5ml	1M
140 mM NaCl	1.4ml	5M
1 mM EDTA pH8	0.1ml	0.5M
1% Triton X-100	2.5ml	20%
0.1% Sodium Deoxycholate	0.5ml	10%
0.1% SDS	0.25ml	20%
Protease Inhibitors (add fresh each time)		

RIPA Buffer	50ml	Stock Solution
50 mM Tris-HCl pH8	2.5ml	1M
150 mM NaCl	1.5ml	5M
2 mM EDTA pH8	0.2ml	0.5M
1% NP-40 (IGEPAL)	5ml	10%
0.5% Sodium Deoxycholate	2.5ml	10%
0.1% SDS	0.25ml	20%
Protease Inhibitors (add fresh each time)		

Wash Buffer	500ml	Stock Solution
0.1% SDS	2.5ml	20%
1% Triton X-100	25ml	20%
2 mM EDTA pH8	2ml	0.5M
150 mM NaCl	15ml	5M
20 mM Tris-HCl pH8	10ml	1M

Final Wash Buffer	500ml	Stock Solution
0.1% SDS	2.5ml	20%
1% Triton X-100	25ml	20%
2 mM EDTA pH8	2ml	0.5M
500 mM NaCl	50ml	5M
20 mM Tris-HCl pH8	10ml	1M

Elution Buffer	10ml	Stock Solution
1% SDS	0.5ml	20%
100mM NaHCO <sub>3</sub>	1ml	1M

**Proteinase K**  
Dissolve in H<sub>2</sub>O at 20 mg/ml, store at -20°C.

At Abcam we use the following reagents:

Compound	Order number	Distributor
Formaldehyde	F-8775	Sigma
Herring sperm ssDNA	ab46666	Abcam
Proteinase K	ab51501	Abcam
Protein A sepharose	17-5280-01	GE Healthcare
Protein G sepharose	17-0618-01	GE Healthcare
Qiaquick PCR Purification kit (250)	28106	Qiagen
Rabbit Control IgG	ab46540	Abcam
RNase A	ab52579	Abcam





## ChIP grade antibodies:

[www.abcam.com/chromatin/chip](http://www.abcam.com/chromatin/chip)

Antibody	Datasheet <a href="http://www.abcam.com/ab...">www.abcam.com/ab...</a>	Clonality	Antibody	Datasheet <a href="http://www.abcam.com/ab...">www.abcam.com/ab...</a>	Clonality	Antibody	Datasheet <a href="http://www.abcam.com/ab...">www.abcam.com/ab...</a>	Clonality	Antibody	Datasheet <a href="http://www.abcam.com/ab...">www.abcam.com/ab...</a>	Clonality
<b>DNA methylation</b>			<b>Histone Modifications (continued)</b>			<b>Modifying enzymes (continued)</b>			<b>Transcription (continued)</b>		
BORIS	P	18337	Histone H3 (di methyl K4) [Y47]	M	32356	HDAC3	P	2379	MAFA	P	17976
Dnmt3b	P	2851	Histone H3 (di methyl K79)	P	3594	HDAC3	P	7030	NCoR2	P	5800
Dnmt3b [52A1018]	M	13604	Histone H3 (di methyl K9) [mAbcam 1220]	M	1220	HDAC3	P	47237	NCoR2	P	24551
MBD1	P	2846	Histone H3 (di+tri methyl K4) [mAbcam 6000]	M	6000	HDAC4	P	1437	NCoR2 [1542]	M	2781
MBD1	P	3753	Histone H3 (mono methyl K27, tri methyl K27 + K4) [8H2]	M	14222	HDAC6	P	47181	NFKB p105 / p50	P	7971
MBD2a	P	3754	Histone H3 (mono methyl K36)	P	9048	KAT13A / SRC1 [1135/H4]	M	84	Nuclear Receptor Corepressor NCoR	P	24552
MBD3	P	3755	Histone H3 (mono methyl K4)	P	8895	KAT13B / AIB1	P	2831	PPAR gamma 1+2 [A3409A]	M	41928
MeCP2	P	2828	Histone H3 (mono methyl K79)	P	2886	KAT13B / AIB1 [AX15.3]	M	2782	RNA polymerase II [H14]	M	24759
MeCP2	P	3752	Histone H3 (mono methyl K9)	P	8896	KAT2B / PCAF	M	12188	RNA polymerase II [H5]	M	24758
<b>Epitope tags</b>			Histone H3 (mono methyl K9)	P	9045	KAT3A / CBP	P	2832	RNA polymerase II CTD repeat YSPTSPS (phospho S2)	P	5095
6X His tag&reg	P	9108	Histone H3 (mono methyl R2)	P	15584	KAT3A / CBP	P	10489	RNA polymerase II CTD repeat YSPTSPS (phospho S5)	P	5131
GAL4	P	1396	Histone H3 (mono+di+tri methyl K79) - Limited Availability and ChIP Grade	P	28940	KAT3A / CBP [AC238]	M	3652	RNA polymerase II CTD repeat YSPTSPS	P	26721
GFP	P	290	Histone H3 (phospho S10) [mAbcam 14955]	M	14955	KAT3B / p300 [3G230]	M	14984	RNA polymerase II CTD repeat YSPTSPS [4H8]	M	5408
HA tag	P	9110	Histone H3 (tri methyl K27) [mAbcam 6002]	M	6002	KAT4 / TBP Associated Factor 1	P	28450	RNA polymerase II CTD repeat YSPTSPS [8WG16]	M	817
HA tag [4C12]	M	1424	Histone H3 (tri methyl K36)	P	9050	KDM1 / LSD1	P	17721	TATA binding protein TBP	P	28175
Maltose Binding Protein [R29.6]	M	65	Histone H3 (tri methyl K4)	P	8580	KDM4A / JHDM2A	P	52002	TATA binding protein TBP [1TB18]	M	12089
Myc tag	P	9132	Histone H3 (tri methyl K4) [mAbcam 1012]	M	1012	KDM5B / PLU1 / Jarid1B	P	50958	TATA binding protein TBP [1TBP18] - Nuclear Loading Control and ChIP Grade	M	818
T7 tag®	P	9138	Histone H3 (tri methyl K4) [mAbcam 12209]	M	12209	KMT1A / SUV39H1 [44.1]	M	12405	TATA binding protein TBP [mAbcam 62126]	M	62126
TFEB	P	2636	Histone H3 (tri methyl K79)	P	2621	LSD2 / AOF1	P	52001	TATA binding protein TBP [mAbcam]	M	51841
V5 tag	P	9116	Histone H3 (tri methyl K9)	P	8898	NCoR2	P	5800	TFEB	P	2636
V5 tag	P	15828	Histone H3	P	1791	NCoR2	P	24551	TFIIB [IIB1]	M	12094
<b>Histone modifications</b>			Histone H3	P	12079	NCoR2 [1542]	M	2781	TFIIE alpha	P	28177
CENPA [3-19]	M	13939	Histone H3	P	46765	Nuclear Receptor Corepressor NCoR	P	24552	<b>Remodelling</b>		
H2A.Z (acetyl K4 + K7 + K11)	P	18262	Histone H3	P	10799	PRMT1	P	3768	BAF53A	P	3882
Histone H1.2	P	4086	Histone H3 [mAbcam 10799]	M	10799	PRMT2	P	3667	RbAp46 / RbAp48 [15G12]	M	490
Histone H2A (acetyl K5)	P	1764	Histone H4 (acetyl K12) - Limited Availability and ChIP Grade	P	1761	PRMT2	P	3763	SMARCA2 / BRM	P	15597
Histone H2A (phospho S129)	P	15083	Histone H4 (acetyl K8)	P	15823	PRMT3	P	3765	SMARCA3	P	17984
Histone H2A	P	15653	Histone H4 (acetyl K91)	P	4627	PRMT6	P	47244	SNF2H	P	3749
Histone H2A	P	18255	Histone H4 (mono methyl K20)	P	9051	RbAp46 / RbAp48 [15G12]	M	490	SUZ12	P	12073
Histone H2A.Z	P	4174	Histone H4 (symmetric di methyl R3)	P	5823	SUZ12	P	12073	RNAi		
Histone H2A.Z	P	18263	Histone H4 (tri methyl K20)	P	9053	<b>Transcription</b>			Chp1	P	18191
Histone H2B (acetyl K5) [EP857Y]	M	40886	Htz1	P	4626	BORIS	P	18337	Dicer [13D6]	M	14601
Histone H2B (acetyl)	P	1759	<b>Modifying enzymes</b>			c-Myc [9E11]	M	56	Other ChIP'ing antibodies		
Histone H2B	P	1790	BAF53A	P	3882	CP2c	P	42973	acetyl Lysine	P	21623
Histone H3 (acetyl K18)	P	1191	CoREST	P	24166	Ctip2 [25B6]	M	18465	Acetylated Proteins	P	193
Histone H3 (acetyl K27)	P	4729	Ctip2 [25B6]	M	18465	FOXA1	P	5089	dimethyl Arginine [21C7]	M	413
Histone H3 (acetyl K9)	P	4441	dHDAC1	P	1767	FOXC1	P	5079	mono and dimethyl Arginine [7E6]	M	412
Histone H3 (acetyl K9)	P	10812	Eaf3	P	4467	GAL4	P	1396	mono methyl Arginine [5D1]	M	415
Histone H3 (acetyl K9)	P	12178	HDAC1	P	7028	HEXIM1	P	25388	pan methyl Lysine (methyl K pan)	M	7315
Histone H3 (acetyl K9) [AH3-120]	M	12179	HDAC1 [1.T.9]	M	31263	Kaiso [6F / 6F8]	M	12723	<b>ChIP-related products</b>		
Histone H3 (acetyl K9, phospho S10)	P	12181	HDAC1 [10E2]	M	46985	KAT13C / NCOA2	P	9261	Herring sperm ssDNA	-	46666
Histone H3 (asymmetric di methyl R17)	P	8284	HDAC1 [6D434]	M	51846	KAT4 / TBP Associated Factor 1	P	28450	Proteinase K protein (Active)	-	51501
Histone H3 (citrulline 2 + 8 + 17) [CitH3]	P	5103	HDAC2	P	7029	KDM5B / PLU1 / Jarid1B	P	50958	Rabbit Control IgG - ChIP Grade	-	46540
Histone H3 (di methyl K27)	P	24684	HDAC2 [3F3]	M	51832	LXR alpha [PPZ0412]	M	41902	RNase A Enzyme protein (Active)	-	52579
Histone H3 (di methyl K4)	P	7766									
Histone H3 (di methyl K4)	P	11946									
Histone H3 (di methyl K4)	P	59563									