

SX-8G IP-Star Automated System Specifications for Auto ChIP

AUTO ChIP			
Factor	Description	Numbers	
Protocol Type	Throughput	8 IPs (1 Peltier block) 16 IPs (2 Peltier blocks)	
	DNA purification method	DNA isolation Buffer (DIB) IPure (DNA magnetic purification)	
	Working volumes	100 µl / 200 µl	
Protocol Duration (including DIB)	Diagenode Optimized Protocols	8 IPs protocol 10h (IP: 5h) 22h (IP: 15h)	16 IPs protocol 22h (IP: 15h; 7.5h mix + 7.5h no mix)
Inputs	Chromatin		
Starting Cell Amount	Fresh cells or frozen fix cells		
Type of Cells		U2OS, MCF7, NB4, HeLa	
Chromatin	Number of cells used	1,000 to 10,000,000 cells	
Min Amount of Chromatin	Min Number of cells	1000 cells	
Max Amount of Chromatin	Max Number of cells	5,000,000 cells	
Chromatin Preparation Conditions	Bioruptor® Sonicator conditions: OPTIMIZATION: for each new cell line, new conditions	Advised starting parameters: Power: "high" Cycles: 10 cycles Time on/off: 30 seconds: "ON" / 30 seconds: "OFF"	
Tubes for shearing	1.5 ml 15 ml	Volumes: 130 µl Max cell number: 10x 10 ⁶ Volumes: 1ml Max Cell number: 30x 10 ⁶	
Fragment Size	Analyze in 1% agarose	100 - 500bp	
Fixation time and conditions		1% Formaldehyde Time: 10 min	
Min Volume for chromatin		100 µl	
Max Volume for chromatin		200 µl	
SDS Concentration during IP	SDS concentration during IP	< 0.15%	
Beads amount	OPTIMIZATION necessary for each antibody (Ab titration)	10 µl/ 3 µg antibody	
Antibody coating Incubation		1-15h	
IP Incubation time	Flexible for customer	1-15h	
IP temperature	Flexible for customer	4°C-25°C	

DNA recovery	Double strand DNA U2OS (1,000,000 cells)	H3K36me3 → 60 ng PottI → 0.91 ER α → 2.37
Test system	Cell line: HeLa cells Antibodies: H3K9me3 and p53 Genomic regions tested: TSH2B (+) and c-Fos (-) with H3K9me3 p21(+) and GADPH(-) with p53	10,000 cells for H3K9me3 1,000,000 cells for p53
Real-time PCR	% recovery; Using Diagenode reagents	> 30% for TSH2B with H3K9me3 3% for p21 with p53 antibody