

## SX-8G IP-Star Automated System Specifications for Auto MeDIP

AUTO MeDIP			
Factor	Description	Numbers	
Protocol Type	Throughput	8 IPs 16 IPs	
	DNA purification method	DNA isolation Buffer (DIB) IPure (DNA magnetic purification)	
	Working volumes	100 µl	
Protocol Duration (including DIB)	Diagenode Optimized Protocols	<b>8 IPs protocol</b> 9h (IP: 5h) 19h (IP: 15h)	<b>16 IPs protocol</b> 19h (IP: 15h; 7.5h mix + 7.5h no mix)
Starting cell amount for shearing		Max cell number: $3 \times 10^6$ / 20 to 30 µg of DNA	
Starting DNA amount for MeDIP		1 µg of DNA	
Type of Cells	U20S, MCF7, NB4		
Cell lysis and DNA purification	GenDNA Module (cell numbers)		
DNA Shearing	Bioruptor® Sonicator conditions	Advised starting parameters: Power: "low" # cycles: 10 cycles Time on/off: 15 seconds: "ON"/ 15 seconds: "OFF"	
Tubes for shearing	1.5 ml	Volumes: 300 µl Amount: 30 µg (0.1 µg/µl)	
	15 ml	Volumes: 1ml Max Cell number: $30 \times 10^6$	
Fragment Size	Analyze in 1% agarose	100 - 500bp	
Starting IP DNA Amount	DNA (0.1 µg/µl)	1 µg	
IP incubation time	Flexible for the customer	1-15 hours	
Antibody	Monoclonal Antibody anti-5meC (cell numbers)	Amount optimized in Diagenode	
DNA internal controls	In vitro Methylated DNA Unmethylated DNA		
Control primers	TSH2B cell specific methylated region GADPH promoter unmethylated region		
DNA recovery after MeDIP	ssDNA – DNA Isolation Buffer ssDNA – IPure (Magnetic Purification)	100 ng (DIB) 80-90 ng (IPure)	
Real-time PCR	% recovery; Using Diagenode reagents (in U20S)	Methylated DNA control → 40% Unmethylated DNA control ← 0.5% TSH2B → 80% GADPH ← 0,5%	

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