



## Chromatin immunoprecipitation from 1.000.000 cells with LowCell# ChIP kit

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The LowCell# ChIP kit was developed to perform chromatin immunoprecipitation from 100.000 to 1000 cells. Application of this kit to the chromatin immunoprecipitation from higher amount of cells – up to 1.000.000 – is also possible.

Nevertheless, few modifications in the original protocol are required when working with 1.000.000 cells. These modifications concern Step 3. Cells lysis and Bioruptor chromatin shearing (point 20, 24) and Step 4. Magnetic Immunoprecipitation (point 29).

Follow Step 1 and 2 described in the manual.

Depending on number of IPs wanted per assay, you will need from 5.000.000 to 10.000.000 of formaldehyde fixed cells.

<b>Total number of cells needed for your ChIP</b>		
<b>Cells per IP</b>	<b>Number of IPs</b>	<b>Cells needed</b>
1.000.000	5 (4 IPs , 1 Input)	5.000.000
1.000.000	10 (9 IPs , 1 Input)	10.000.000

Proceed to Step 3, points 17, 18, 19 as described in the manual.

**Attention: following points are different from the manual**

20. Resuspend 5.000.000 cells in 130 µl of complete buffer B. Incubate on ice for 10 min. If chromatin from more than 5.000.000 is required, resuspend additional 5.000.000 cells in 130 µl.

*Do not resuspend more than 5.000.000 cells in 130 µl, otherwise the cell lysis and chromatin shearing will be inefficient*

21. Submit the samples to sonication to shear chromatin using Bioruptor for 12-15 cycles of 30' ON/OFF at high power.

*Depending on cell type, additional optimization of shearing might be needed.*

22. Centrifuge samples at 14000 rpm during 10 min at 4°C. Keep the supernatant on ice.

*The centrifugation is a critical to eliminate cell debris, otherwise it will result in high background. This step is not necessary when working with low amount of cells.*

23. Prepare complete buffer A as described in the manual

24. Add 620 µl of complete buffer A to 130 µl of sheared chromatin. Use 150 µl of diluted chromatin (corresponding to 1 million cells) per one IP (Step 4, point 29).

*Do not use more than 150 µl of sheared chromatin per IP in order to insure good mixing*

Proceed to the Next Steps (4, 5 and 6) as described in the manual.