Services ChIP-Seq Double Fixation Protocol (DSG + Formaldehyde) for Cells

Active Motif Services requires a minimum of 4-5 million cells per IP. However, more cells are encouraged and ≥10 million can be beneficial for transcription factors. Fix ALL cells in a population at once, scaling up as required.

Required Reagents and Materials:

- DSG, disuccinimidyl glutarate (*e.g.* Thermo Scientific™ Pierce™ Cat No. A35392 or 20593, MW 326.26)
- DMSO, anhydrous dimethyl sulfoxide (*e.g.* ThermoFisher Scientific™ Invitrogen™ Cat No. D12345)
- PBS, phosphate-buffered saline without Ca^{2+}/Mg^{2+} (*e.g.* ThermoFisher Scientific™ Gibco™ Cat No. 10010023)
- 37% formaldehyde solution (*e.g.* Sigma-Aldrich Cat No. F8775)
- Glycine (e.g. Sigma-Aldrich Cat No. G7403, MW 75.07)
- 10% BSA Solution, Nuclease-Free (*e.g.* Sigma-Aldrich Cat No. 126615)
- 1.5 ml, 15 ml, and 50 mL centrifuge tubes
- Dry ice

Prepare Buffers

Prepare buffers below according to the number of cells being fixed. The tables have been provided for convenience.

2 mM DSG Solution

Prepare **fresh** 2 mM DSG Solution by first creating a 0.5 M DSG stock solution in DMSO and then diluting this into PBS.

0.5 M DSG Stock	**10 million cells**	**75 million cells**
DSG (MW 326.26)	6.53 mg	50 mg
DMSO	Fill to 40 µL	Fill to 300 µL

2 mM DSG Solution	**10 million cells**	**75 million cells**
0.5 M DSG Stock	40 µL	300 µL
PBS	10 mL	75 ml

37% Formaldehyde Solution

28 µL of 37% Formaldehyde Solution is required per 1 million cells or each 1 ml of cell suspension.

2.5 M Glycine Solution

Prepare 2.5 M Glycine Solution by adding glycine to a 1.5 ml or 15 ml conical tube and adding water to the indicated volume. Place at room temperature.

2.5 M Glycine Solution	*10 million cells*	*75 million cells*
Glycine (MW 75.07)	101 mg	760 mg
H_2O	Fill to 540 µL	Fill to 4.05 ml

0.5% BSA Solution

Prepare 0.5% BSA Solution by mixing the indicated volumes of 10% BSA and PBS in a vessel. Place at 4°C.

0.5% BSA Solution	*10 million cells*	*75 million cells*
10% BSA	1.0 mL	7.5 mL
PBS	19 mL	142.5 mL
Total	20 mL	150 mL

Protocol:

1. Starting with the cell pellet, add 1 mL of 2 mM DSG Solution per 1 million cells. Mix thoroughly via pipetting up and down and incubate the cell suspension for 30 minutes at room temperature (RT) in an orbital shaker. Start the timer as soon as the last tube has received the 2 mM DSG Solution.
2. For each mL or 1 million cells add 28 µL of 37% formaldehyde to create a final concentration of 1% formaldehyde. For example, if you have fixed 10 million cells in 10 ml, add 280 µL of 37% formaldehyde. Mix well and incubate for 10 minutes at room temperature in an orbital shaker. Start the timer once the last tube has received the formaldehyde.
3. Per each mL or 1 million cells add 54 µL 2.5 M glycine to create a final concentration of 0.125 M glycine to quench the fixation reaction. For example, for 10 mL of cell suspension add 540 µL of 2.5 M glycine per mL of solution.
4. Centrifuge at 1000 x *g* for 5 minutes at 4°C. Remove supernatant.

   Note: Formaldehyde is hazardous and should be disposed of according to local hazardous waste regulations.

5. Wash cells by doing the following:

1) Per 1 million cells add 1 mL of cold 0.5% BSA Solution.
2) Pipette up and down.
3) Centrifuge at 1000 x *g* for 5 minutes at 4°C.
4) Remove supernatant.

6. Wash a second time:
    1) Per 1 million cells add 1 mL of cold 0.5% BSA Solution.
    2) Pipette up and down.
    3) If the cells from any one population are contained in multiple tubes, combine them into one tube at this point.
    4) Centrifuge at 1000 x *g* for 5 minutes at 4°C.
    5) Remove supernatant.
7. Snap freeze the cell pellet on dry ice and store at -80°C.

Best Practices for sending samples to Active Motif

- Seal top of tube with parafilm to avoid tube from opening during transit
- Ensure that there is enough dry ice in package for transport
- Avoid shipping over a weekend or for Saturday delivery
- Ship samples Monday through Wednesday
- Ensure that a complete sample submission form is included in the shipment