**JARID1CA-p054**

**Growth method**:

Medium:

Virus amplification: Sf900 III (Gibco) + 2% FBS.

Expression: Insect-Xpress (Lonza).

6 x 1L of Sf9 insect cells in 3L glass non-baffled flasks were infected with 3mL of virus P2 per flask.Cell density at infection time: 2e6 /mL. Protein was expressed for 72h at 27°C with 100rpm shaking.

**Extraction buffers**:

Lysis buffer: 50mM HEPES-KOH, 0.3M KCl, 5% glycerol, 10mM imidazole pH 7.4, protease inhibitors cocktail set VII (Calbiochem) and 250U of benzonase (EMD Milipore)

**Extraction procedure**:

Fresh cell pellets were resuspended in a total volume of 120 mL of lysis buffer. Cells were broken by dounce homogenisation, approx 30 strokes. Cell debris were removed by centrifugation for 60 minutes at 21krpm.

**Purification buffers**:

Lysis: 50mM HEPES-KOH, 0.3M KCl, 5% glycerol, 10mM imidazole pH 7.4

Wash: 50mM HEPES-KOH, 300mM KCl 5% glycerol, 30mM imidazole

Elution: 50mM HEPES-KOH, 300mM KCl, 5% glycerol, 250mM imidazole

Gel filtration: 50mM HEPES-KOH, 0.3M KCl, 5% glycerol pH 7.4

**Purification procedure**:

Column 1: Ni-affinity, HisTrap FF Crude, 5 mL (GE/Amersham Biosciences).The cell extract was loaded on the column at 3 mL/minute on an AKTA-express system (GE/Amersham). The column was then washed with 20 column volumes of lysis buffer, and 10 volumes of wash buffer and then eluted

with elution buffer. The eluted peak of A280 was automatically collected.

Column 2: Gel Filtration, Hiload 16/60 Superdex 200 prep grade, 120 mL (GE/ Amersham Biosciences).The eluted fractions from the Ni-affinity Histrap column were loaded on the gel filtration column at 0.8 mL/min. Eluted proteins were collected in 1.8 mL fractions. The main peak not containing aggregates was pooled.

Purification procedure has to be performed during one day due to fastdegradation and loss of activity.

**Protein stock concentration**:

The protein was concentrated using an Amicon Ultracel centrifugal concentrator (50 kDa MWCO) to 4.6 mg/ml by A280 and extinction coefficient. Aliquots were snap frozen and kept in -80°C.

**Mass spec**:

The molecular weight of protein couldn't be evaluated by Mass spectroscopy due to its size of 91kDa. Protein migrates correctly on SDS-page and shows specific demethylase activity by alpha screen assay.