**Expression and Purification of Sirt3 (102-399) from Arctic Express (DE3) cells:**

1. Overexpression of Sirt3 (102-399):

* Pick a single isolated colony from a freshly transformed LB-ampicillin (100ug/ml) plate (or frozen glycerol culture stock) and inoculate into 3 ml LB-amp. Grow at 37 C, 250 rpm for 16 hrs (overnight).
* Inoculate 3 ml overnight culture into 200 ml LB medium (no antibiotics). Grow at 30 C, 250 rpm for 4 hrs.
* After 4 hrs of growth at 30 C, lower temperature to 15 C and continue growing for 30 mins to equilibrate the cultures to 15 C.
* After 30 min equilibration at 15 C, induce Sirt3 overexpression by adding 1 mM IPTG (final concentration).
* Continue growing the cultures for 24 hrs at 15 C, 250 rpm.
* Harvest the cells by centrifugation at 6000 x g for 20 min at 4 C.
* Discard the supernatant and store the cell pellets in -80 C.

1. Purification of Sirt3 (102-399) using 1 ml His-Gravitrap columns:

* Thaw cell pellets on ice, resuspend pellets in minimal volume of LEW 1X buffer (50 mM NaH2PO4, 300 mM NaCl, 10 mM Imidazole, pH 8.0).
* Sonicate the resuspended cells with 5 pulses, 5 sec each, with 1 min incubation on ice between each pulse.
* Centrifuge the sonicated sample at 14,000 x g for 20 min at 4 C.
* Load the supernatant on to a 1 ml His-Gravitrap column, pre-equilibrated with LEW 1X buffer.
* Collect the flowthrough and reload. Repeat 3x.
* Wash the column with 10 column volumes (cv) LEW 1X buffer.
* Wash the column with 10 cv Wash 1 buffer (LEW 1X with 75 mM Imidazole, pH 8.0).
* Wash the column with 10 cv Wash 2 buffer (20 mM Tris-HCl, 2M Urea, pH 6.8).
* Wash the column with 10 cv Wash 1 buffer.
* Elute Sirt3 as 300 ul fractions using Elution buffer (LEW 1X with 300 mM Imidazole, pH 8.0).
* Verify the strongest elutions by SDS-PAGE.
* \*\* For one 1 ml His-Gravitrap column, using 1 batch of cells (200 ml), strongest elution bands = elutions 4,5,6,7.
* Pool the selected fractions (~1.2 ml) and dialyze overnight in 1 L Dialysis buffer (25 mM Tris, pH 7.5, 100 mM NaCl, 5 mM DTT, 10 % glycerol) at 4 C.
* Next morning, mix the dialyzed Sirt3 thoroughly by pipetting and store as 50 ul aliquots in -80 C.

Buffers:

1. Lysis-Equilibration-Wash (LEW 1X) buffer: 50 mM NaH2PO4, 300 mM NaCl, 10 mM Imidazole, pH 8.0
2. Wash 1 buffer: LEW 1X with 75 mM Imidazole, pH 8.0
3. Wash 2 buffer: 20 mM Tris-HCl, 2M Urea, pH 6.8
4. Elution buffer: LEW 1X with 300 mM Imidazole, pH 8.0
5. Dialysis buffer: 25 mM Tris, pH 7.5, 100 mM NaCl, 5 mM DTT, 10 % glycerol