Protein information sheet

Gene name	**GLE1**
Uniprot ID	Q53GS7
Region	Q383 – S698

Description	RNA export mediator
Synonyms	GLE1L, LCCS, LCCS1, hGLE1
Construct ID	GLE1A-c200
Protein sequence	*QDITMQWYQQLQDASMQCVLTFEGLTNSKDSQAKKIKMDLQKAATIPVSQIST IAGSKLKEIFDKIHSLLSGKPVQSGGRSVSVTLNPQGLDFVQYKLAEKFVKQG EEEVASHHEAAFPIAVVASGIWELHPRVGDLILAHLHKKCPYSVPFYPTFKEG MALEDYQRMLGYQVKDSKVEQQDNFLKRMSGMIRLYAAIIQLRWPYGNRQEIH PHGLNHGWRWLAQILNMEPLSDVTATLLFDFLEVCGNALMKQYQVQFWKMLIL IKEDYFPRIEAITSSGQMGSFIRLKQFLEKCLQHKDIPVPKGFLTSSFWRSAS GLNDIFEAQKIEWHE*

Protein mass	with biotin, + 226.1	Protein pI	Extinction (M^{-1} cm^{-1})
38167.2	38393.2	8.24	58900

Expression host	E. coli BL21(DE3)-R3-BirA
Expression medium	Terrific broth (TB) with 50 µg/ml kanamycin, 50 µg/ml Streptomycin and Chloramphenicol (34 µg/ml) included in starter cultures but not in final growth. Biotin (10 mM stock): Add 24mg of d-biotin to 10 ml of warm (microwaved) 10mM bicine buffer (pH 8.3). Sterilize the solution with a syringe and a 0.2 micron filter.
Transformation and storage	Cells were transformed with the expression plasmid and selected on LB-agar plate's containing kanamycin, streptomycin and chloramphenicol. Several colonies were picked from the overnight transformation and used to inoculate cultures in LB supplemented with KAN/Cam/Str which were grown overnight and stored as glycerol (15% v/v) stock at -80°C.
Expression and *in vivo* biotinylation	10ml starter cultures in LB supplemented with KAN/Cam/Str were grown overnight at 37°C with shaking at 250rpm. Next day, 10 ml was used to inoculate 1L of TB in a 2LUltraYield flask supplemented with Streptomycin 50µg/ml and Kanamycin 50µg/ml. **Note:** the medium need not contain chloramphenicol at this step; the pRARE2 plasmid is not lost during the growth process and also ensures a more predictable growth. Seal flask with an adhesive air porous film during cell growth. Culture was grown in a 37°C incubator with shaking at 200rpm. Cell growth was monitored until Mid-log phase was reached and cell density OD_{600nm} was approximately 2.00 – 3.00 in TB. Incubator temperature was lowered to 18ºC and culture flask allowed to cool. After 30 - 45 minutes protein expression was induced

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	by the addition of IPTG at 0.1mM final concentration. During the induction process Biotin solution was added in appropriate concentrations. 10ml of biotin solution is added to each 1L culture on induction with IPTG. Incubation of culture was continued for 16 hours at 18°C. Next day, the cells were collected by centrifugation (15 min, 5,000 RPM in JLA-8.100 rotors). The supernatant discarded and the cell pellet transferred into a 50ml conical polypropylene tube. Cell pellet was weighed, frozen and stored at -80°C.
Purification buffers	2x lysis buffer: 100 mM HEPES, pH 7.5, 1.0 M NaCl, 10% v/v glycerol, 1mM TCEP, 10mM imidazole. Protease inhibitors (1:200 Merck cocktail IV) were added before use. 1x lysis buffer: 50 mM HEPES, pH 7.5, 0.5 M NaCl, 5% v/v glycerol, 1 mM TCEP, 10 mM imidazole Wash buffer: 50 mM HEPES, pH 7.5, 0.5 M NaCl, 5% v/v glycerol, 1 mM TCEP, 40 mM imidazole Elution buffer: 50 mM Hepes pH 7.5, 500mM NaCl, 5% Glycerol, 1mM TCEP, 100mM, 150mM, 250 mM, 500 mM Imidazole, respectively GF buffer: 50 mM HEPES, pH 7.5, 0.5M NaCl, 5% v/v glycerol, 1 mM TCEP PEI 5%, pH 7.5: 5% polyethyleneimine (diluted from 50% stock, Sigma) and **titrated to pH 7.5 with HCl.**
Purification step 1: IMAC	**Cell Lysis:** Frozen cell pellets from -80°C were thawed by intermittently placing in a 37°C water bath, then transferred directly to ice. Cells were re-suspended in pre-chilled lysis buffer by vortex or pipetting gentle until cells were completely in suspension. 1 volume (v/w) of 2x lysis buffer was added, and the cells were thoroughly suspended. After further addition of 3 volumes (3 ml per 1g of pellet) of 1x lysis buffer the cells were broken by sonication. Lysate was then transferred to a 50ml Beckman polypropylene centrifuge tube. Cell debris and DNA from the lysate was precipitated by adding Polyethylenimine (PEI) to the tube from a 5% stock solution to a final concentration of 0.15% and incubated on ice for 15 – 30 mins for precipitation. Cell lysates were clarified by centrifugation using a JA25.50 rotor at 21,500rpm for 60 min at 4°C. The cleared supernatant was collected and filtered using a 0.45um acrodisc syringe filter and transferred to a fresh 50ml-conical polypropylene tube. **Ni^{2+}-IDA IMAC (column 1):** A 25ml Econo-Pac polypropylene column (BioRad) was packed with 5ml of Ni-Sepharose resin and equilibrated in 1x lysis buffer. The cell lysate was loaded onto the column and passed through resin at gravity flow. The unbound flow-through was collected, a wash step of 10 column volumes of wash buffer was included and collected as the wash fraction. Protein was eluted in four intermediate fractions with a step gradient of 5ml imidazole elution buffers; 100mM, 150mM, 250mM and 500mM imidazole, respectively, each gradient eluted fraction was collected. IMAC fractions were analysed by SDS-PAGE gel for expected

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	molecular weight and purity, the selected eluted fractions were pooled together. **Mass spectrometry characterisation:** Identity and biotinylation efficiency was verified by mass spectrometry, using a Quadrupole-Time of flight (Q-TOF) analyser, Biotin quantitation is measured by: MW of biotinylated protein = MW of sequenced Protein + 226 Da (biotinylation modification).
Step 2: Removal of His_6 tag and Ni^{2+}-IDA reverse binding	**Enzymatic removal of histidine tag:** Selected, pooled eluted fractions from Ni^{2+}-IDA IMAC were concentrated using a 30,000 Amicon-Ultra MWCO centrifugal concentrator to approximately 10mls in volume. Cleavage of the histidine tag was performed by the addition of SUMO protease in a 1:20 w/w ratio to the pooled eluted protein fractions. The protein sample plus SUMO protease was transferred to Snakeskin dialysis tubing (3,500 MWCO) and dialysed in 1L Gel Filtration buffer overnight at 4°C on a magnetic stirrer plate for digestion. Next day, the protein was applied to a Ni^{2+}-IDA reverse-binding column. **Ni^{2+}-IDA Rebinding column (Column 2):** Separate cleaved protein from any un-cleaved protein by passing over a Ni^{2+}-IDA column. Cleaved protein may be recovered in the flow-through fractions. Non-specific contaminating proteins with any un-cleaved protein bound to the column can be eluted with imidazole. To a 25ml Econo-Pac polypropylene column (BioRad), 2ml of packed Ni-Sepharose resin was added, resin was washed in 3 column volumes of distilled deionized water then equilibrated in 5 column volumes of gel filtration buffer. The overnight protein plus SUMO protease sample was loaded and passed through the column by gravity flow. The flow through was collected, to wash any remaining unbound protein from the column a 2x wash step of 2 column volumes of gel filtration buffer was included. Any bound protein was eluted with 2 column volume washes of 40 mM and 500mM imidazole elution buffer. Collected fractions were analysed for verification of cleaved protein and purity by SDS-PAGE gel and mass spectrometry characterisation. Observation of SDS-PAGE gel analysis identified two protein bands after column 2 in the sample flow-through, GF buffer washes 1 and 2 and 40 mM Imidazole wash. Band 1 at the expected molecular weight in the region of 38kDa, band 2 within the 35kDa region. Mass spectrometry results for GLE1 verified the expected mass with biotinylation plus cleavage of the histidine tag to be 38393Da, a second mass of 35412 was also found to be present within the sample. Tandem MS/MS was performed on cut gel bands for identification of the second observed mass at 35412, results were shown to determine the mass to be BIRA_ECOLI (UniProt: P06709). BirA ligase is known to sometimes co-purify with the target biotinylated protein. The flow-through, GF buffer washes and 40 mM fractions from the Ni^{2+}-IDA rebinding column were pooled and prepared for GF-SEC, in an attempt to recover GLE1 protein only with un-bound BirA ligase.
Step 3:	

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SEC: Gel filtration	**Size Exclusion Chromatography (SEC) (Column 3):** Verified protein from rebinding column 2 was concentrated to required volume for gel filtration (<5 ml), using an Amicon-Ultra 30,000 MWCO centrifugal concentrator. The GF column (Superdex S200 16/60 HiLoad, 120ml on an ÄKTA Express chromatography system) was equilibrated in gel filtration buffer. The concentrated protein sample was loaded onto the GF column for further purification; protein was fractionated on the column in GF buffer at 1ml/min and 1ml fractions were collected at the A280 peaks. From evaluation of the GF chromatogram two protein peaks were observed, both peak fractions were analysed by SDS-PAGE gel and mass spectrometry. Both biotinylated GLE1 and BirA proteins were incorporated into GF peak 1, represented by double bands on SDS-PAGE, with a 50% ratio of each as characterised by mass spectrometry. GLE1 protein was observed as a single band in GF peak 2 after the third fraction into the peak, as analysed by SDS-PAGE. Fractions containing only a single band were pooled and verified using mass spectrometry as one protein species and of the correct mass with 100% biotinylation without any post-translational modifications.
Step 4: Protein storage	**Protein concentration mg/ml and storage:** Protein was concentrated using a 30,000 Amicon-Ultra MWCO centrifugal concentrator and final protein concentration was determined from the absorbance at 280nm, measured using a nanodrop spectrometer (Nanodrop ND-1000, NanoDrop Technologies). Final protein concentration was measured at 1.7mg/ml with a final volume of 1ml. Protein was transferred into 200 µl aliquots, flash-frozen in liquid nitrogen and stored at -80°C.

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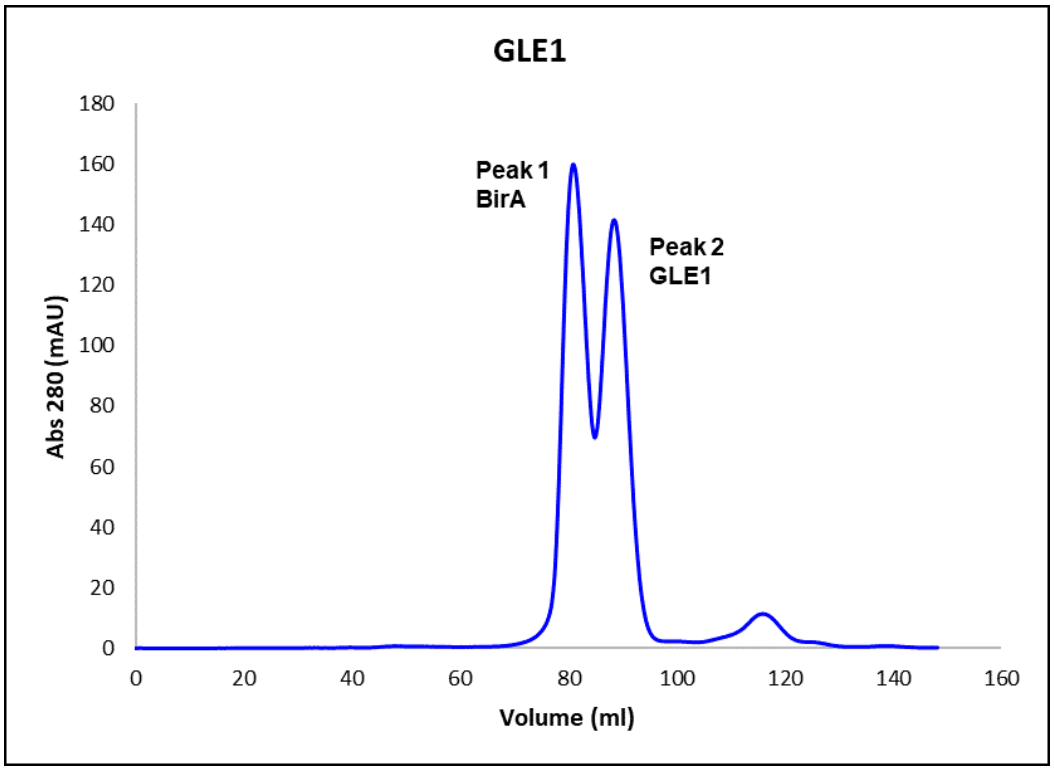
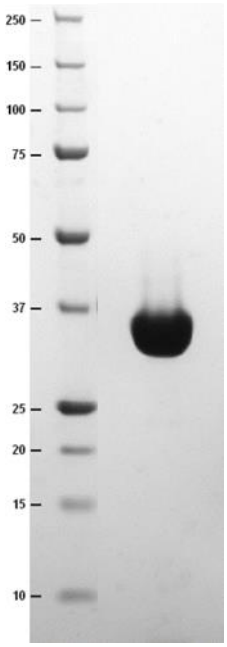
Gel Filtration Chromatogram (Superdex S200)



GLE1A-c200, Maximum peak elution time was 88.354ml with absorbance at 280nm of 141.459 mAU. SEC analysis validates protein is a monomer.

Final Sample SDS-PAGE



Protein	Expected mass + Biotinylation (Da)	Final concentration (mg/ml)
GLE1A-c200	38393.2	1.7

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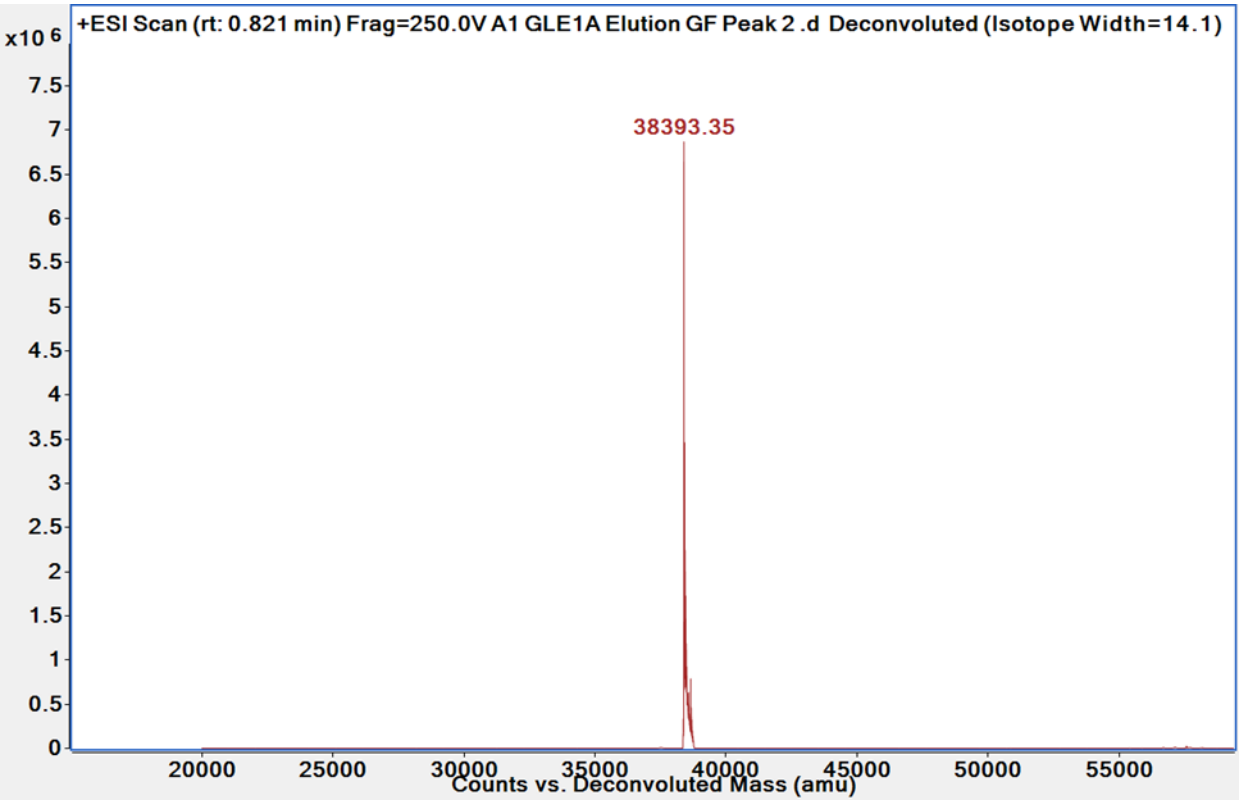
Mass Spectrometry characterisation

Protein	Vector	Expected mass cleaved + Biotinylation (Da)	Observed mass	Comments
GLE1A-c200	pSUMO-LIC	38393.2	38393.35	100% Biotinylated



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