Protein information sheet

Gene name	**MATR3**
Uniprot ID	P43243
Region	M389 - R578

Description	Matrin 3
Synonyms	ALS21, MPD2, VCPDM.
Construct ID	MATR3A-c031
Protein sequence	*SM*QKGRVETSRVVHIMDFQRGKNLRYQLLQLVEPFGVISNHLILNKINEAFIE MATTEDAQAAVDYYTTTPALVFGKPVRVHLSQKYKRIKKPEGKPDQKFDQKQE LGRVIHLSNLPHSGYSDSAVLKLAEPYGKIKNYILMRMKSQAFIEMETREDAM AMVDHCLKKALWFQGRCVKVDLSEKYKKLVLRS*SKGGYGLNDIFEAQ**K**IEWHE*

Protein mass	**with biotin, + 226.1**	**Protein pI**	**Extinction (M^{-1} cm^{-1})**
24491.45	24717.5	9.41	24410

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) and sterilize of 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 by the addition of IPTG at 0.1mM final concentration. During the induction

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	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 JLS-8.100 rotors). The supernatant discarded and th(10 mM)e 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, 10% v/v glycerol, 1 mM TCEP, 10 mM imidazole Wash buffer: 50 mm HEPES, pH 7.5, 0.5 M NaCl, 10% v/v glycerol, 1 mM TCEP, 40 mM imidazole Elution buffer: 50mM Hepes pH 7.5, 500mM NaCl, 10% Glycerol, 1mM TCEP, 100mM, 150mM, 250Mm, 500 Imidazole, respectively GF buffer: 20 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, the column was washed with 10 column volumes (CV) of wash buffer, which was collected as the wash fraction. Protein was eluted in four intermediate fractions with a step gradient of 5ml each of elution buffers containing 100mM, 150mM, 250mM and 500mM imidazole, respectively, each gradient eluted fraction was collected. IMAC fractions were analysed by SDS-PAGE gel for expected 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

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	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 10,000 Amicon-Ultra MWCO centrifugal concentrator to approximately 10mls in volume. Cleavage of the histidine tag was performed by the addition of His_8-taggedTEV protease in a 1:20 w/w ratio to the pooled eluted protein fractions. The protein sample plus TEV 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. **Ni^{2+}-IDA Rebinding column (Column 2):** To remove uncleaved protein and other contaminants, the dialysate was passed through a second IMAC column. 1ml of packed Ni-Sepharose resin was added to a 25ml Econo-Pac polypropylene column (BioRad), washed in 3 CV of distilled deionized water then equilibrated in 5 column volumes of gel filtration buffer. The cleaved, dialyzed protein sample was loaded and passed through the column by gravity flow. The flow through was collected and combined with two 2 CV washes in GF buffer gel filtration buffer. For completeness, the column was finally eluted with a 2 CV wash of 500mM imidazole elution buffer. All fractions were analysed for verification of cleaved protein and purity by SDS-PAGE gel and mass spectrometry characterisation.
Step 3: SEC: Gel filtration	**Size Exclusion Chromatography (SEC) (Column 3):** Verified protein from rebinding column 2 was concentrated to less than 5 ml, using an Amicon-Ultra 10,000 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 0.5ml fractions were collected at the A280 peaks. Peak fractions were analysed by SDS-PAGE gel, selected fractions containing high protein concentration and purity were collected and pooled, eliminating any bound contaminating non-specific proteins.
Step 4: Protein storage	**Protein concentration mg/ml and storage:** Protein was concentrated using a 10,000 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 2.67mg/ml with a final volume of 5mls. Protein was transferred into 200 µl aliquots, flash-frozen in liquid nitrogen and stored at -80°C. The final storage buffer was 20mM Hepes, pH 7.5, 500mM NaCl, 5% v/v glycerol, 1 mM TCEP.

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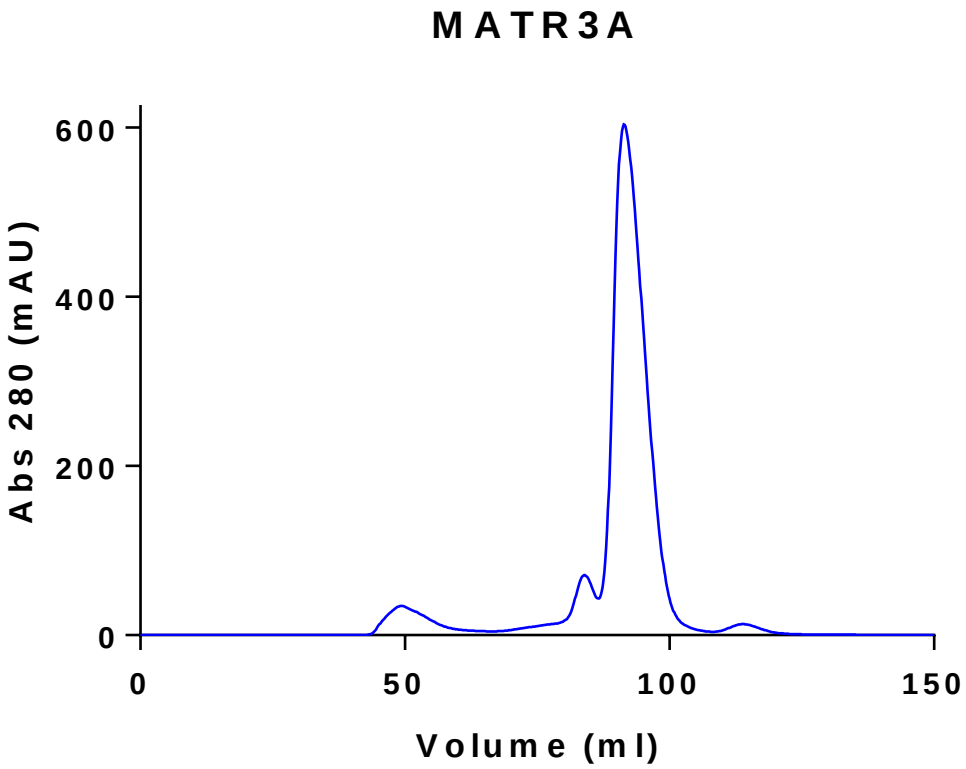
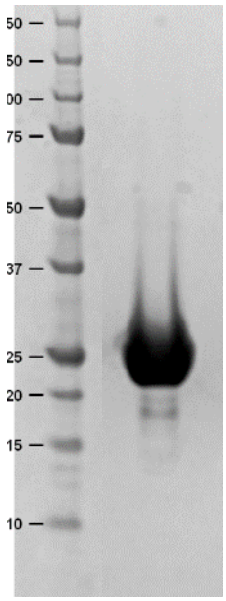
Gel Filtration Chromatogram (Superdex S200)



MATR3A-c031, Maximum peak elution time was 91.4 ml with A 280 of 603.847 mAU. SEC analysis validates protein is a monomer.

Final Sample SDS-PAGE



Protein	Expected mass + Biotinylation (Da)	Final concentration (mg/ml)
MATR3A-c031	24717.5	2.67

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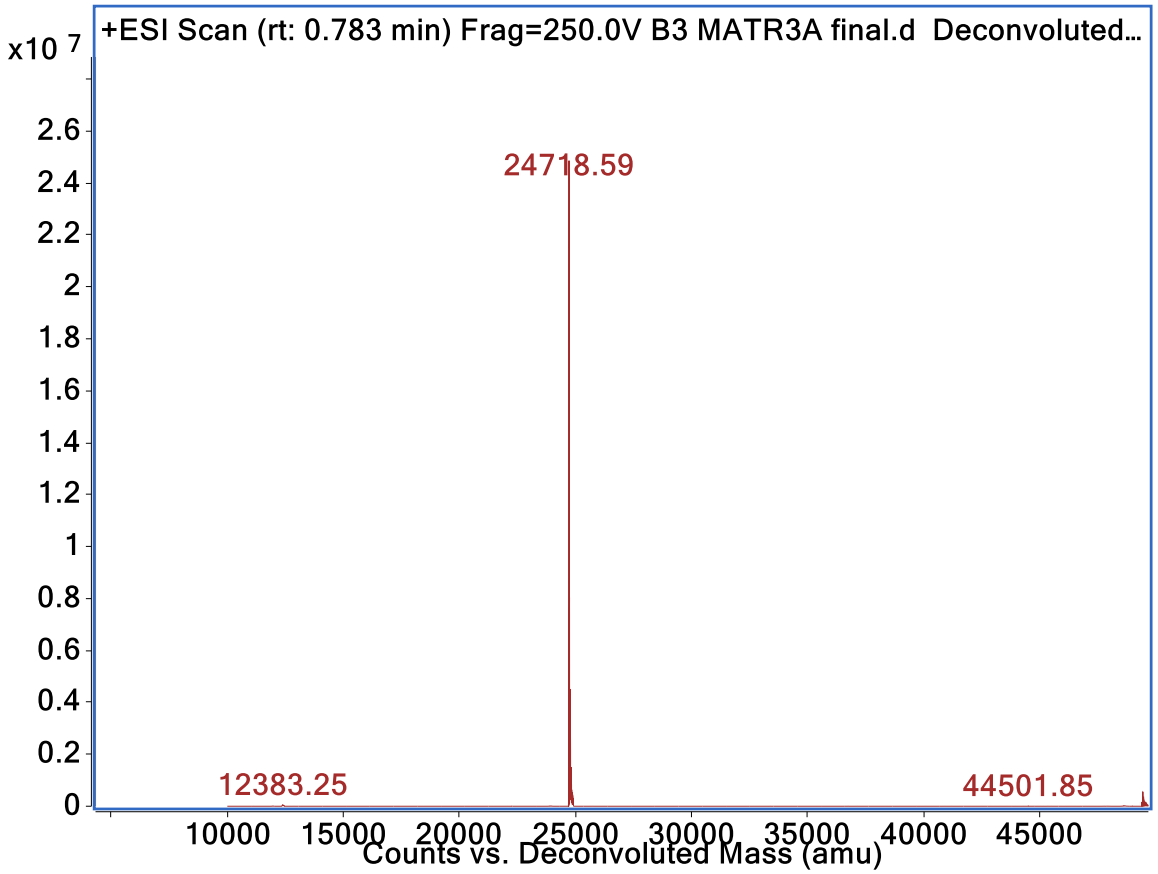
Mass Spectrometry characterisation

Protein	Vector	Expected mass cleaved + Biotinylation (Da)	Observed mass	Comments
MATR3A-c031	pNIC-Bio3	24,718	24,718	100% biotinylated



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