

Supplementary Materials

Revisiting the potential functionality of the MagR protein

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1. Amino acid sequences of target proteins

MagR from *Columbia livia* (cIMagR)

XP_005508102.2 reference

MPAAVPGLCGLRRGTSPHMVPAVPGTLVWTPSAVQKIKELLKDKPEHVGKVGVRTRGCNGLSYTLE
YTKSKGDSDEEVVQDGVRFIEKKAQLTLLGTEMDFVEDKLSSEFVFNNPNIKGTCGCGESFNI

Native amino acid sequence of expressed cIMagR protein

PAAVPGLCGLRRGTSPHMVPAVPGTLVWTPSAVQKIKELLKDKPEHVGKVGVRTRGCNGLSYTLEY
TKSKGDSDEEVVQDGVRFIEKKAQLTLLGTEMDFVEDKLSSEFVFNNPNIKGTCGCGESFNI

Native amino acid sequence of expressed cIMagR-his protein

PAAVPGLCGLRRGTSPHMVPAVPGTLVWTPSAVQKIKELLKDKPEHVGKVGVRTRGCNGLSYTLEY
TKSKGDSDEEVVQDGVRFIEKKAQLTLLGTEMDFVEDKLSSEFVFNNPNIKGTCGCGESFNILEHHHH
HH

MagR from *Drosophila melanogaster* (dMagR)

NP_573062.1 reference

MATRVVATATVRAVKGRKLIPTRAALTTPAAVLRIKTLLQDKPDMVGLKVGVRQRGCNGLSYTLDY
ASQKDKLDEEVVQDGVKVFIDKKAQLSLLGTEMDFVESKLSSEFVFNNPNIKGTCGCGESFSM

Native amino acid sequence of expressed dMagR protein

ATRVVATATVRAVKGRKLIPTRAALTTPAAVLRIKTLLQDKPDMVGLKVGVRQRGCNGLSYTLDYA
SQKDKLDEEVVQDGVKVFIDKKAQLSLLGTEMDFVESKLSSEFVFNNPNIKGTCGCGESFSM

Native amino acid sequence of expressed dMagR-his protein

ATRVVATATVRAVKGRKLIPTRAALTTPAAVLRIKTLLQDKPDMVGLKVGVRQRGCNGLSYTLDYA
SQKDKLDEEVVQDGVKVFIDKKAQLSLLGTEMDFVESKLSSEFVFNNPNIKGTCGCGESFSMLEHHHH
HH

2. Plasmid sequences from GenScript with target genes

pET-21a(+) with dMagR (target with start and stop codon is underlined)

5'-

tggcgaatgggacgcgccctgtagcggcgacgaatgaagcgccgggtgtggtgttacgcgcagcgtgaccgctacacttgccagcgccctagcgcggcgtcctt
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tttttctgaaaggaggaaactatataccgat-3'

pET-21a(+) with dMagR-his (target with start and stop codon is underlined)

5'-

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tctgaaaggaggaactatatccggat-3'

pET-21a(+) with cIMagR (target with start and stop codon is underlined)

5'-

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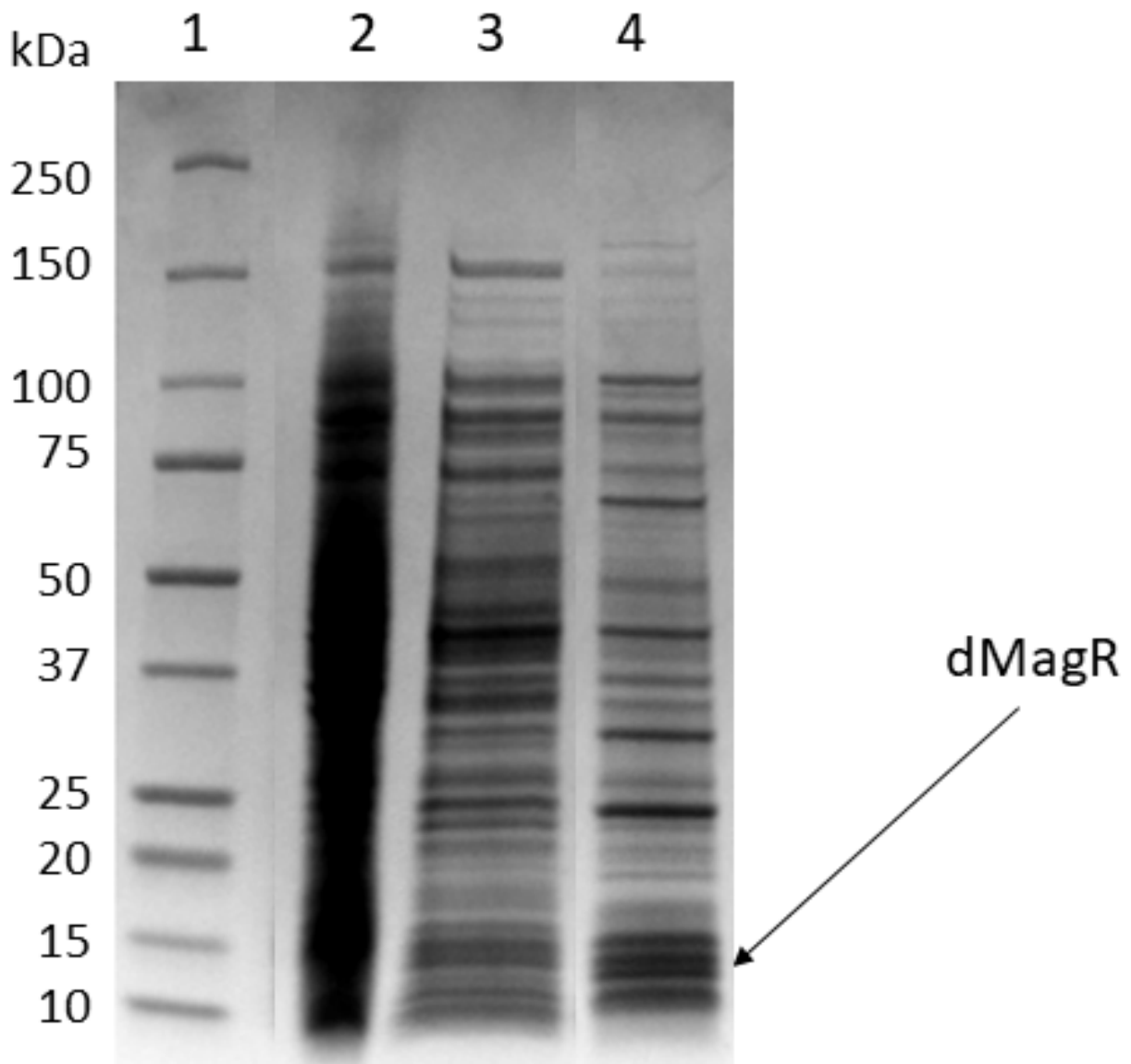
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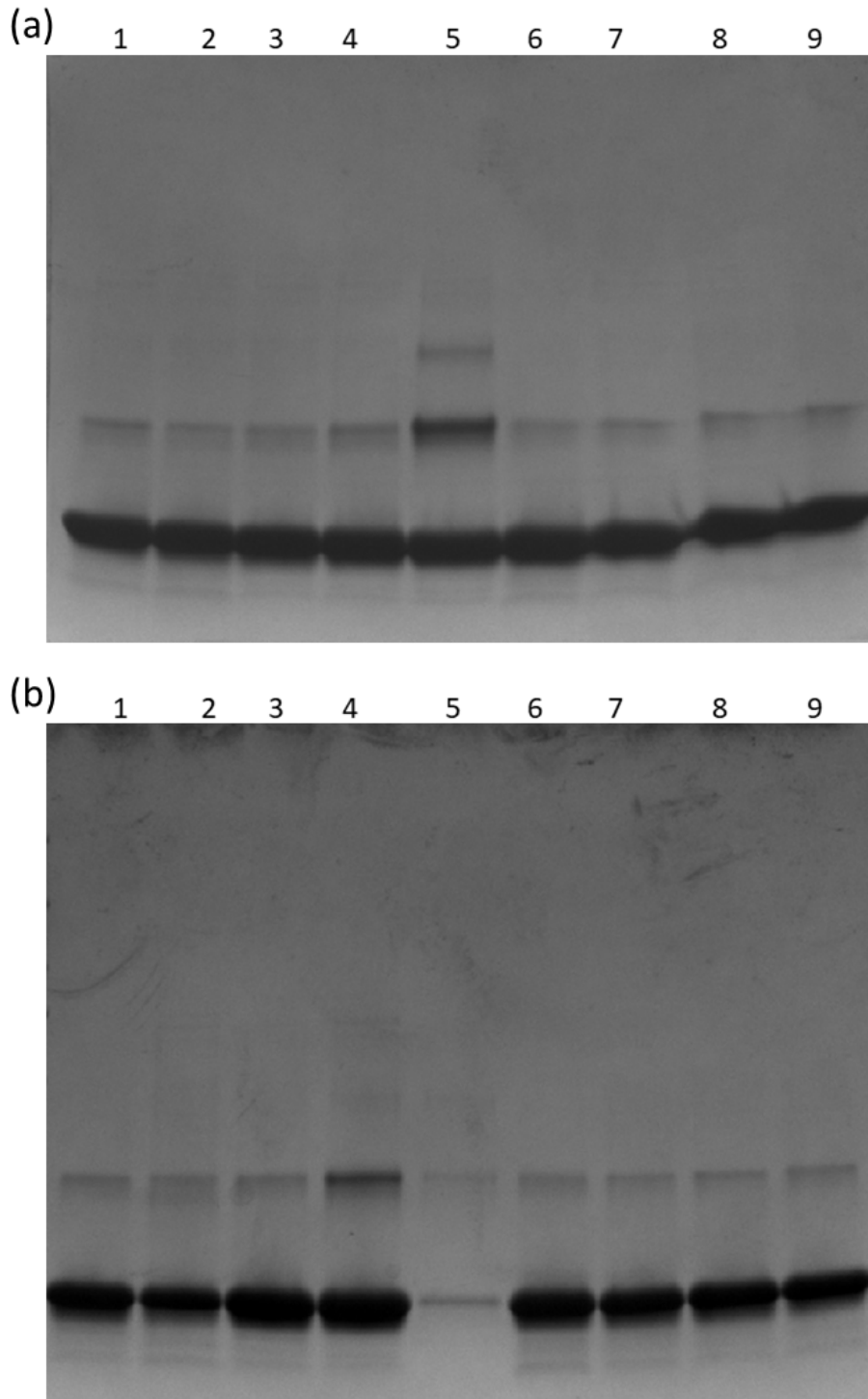
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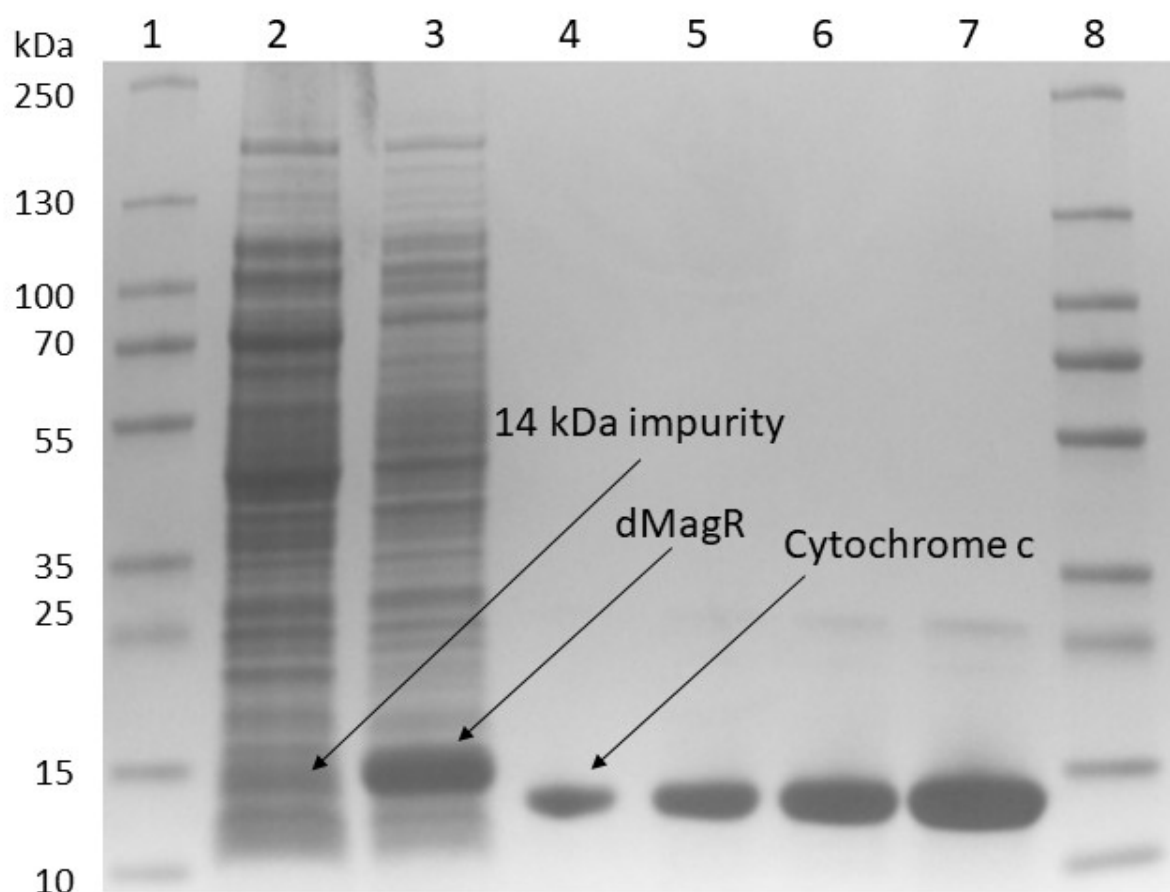
3. Additional SDS-PAGE investigations



Supplementary Figure S1. SDS-PAGE analysis to test dMagR capture with beads. lane 1: standard protein ladder; lane 2: 4 μ L solubilized cell pellet of BL21-dMagR lane 3: supernatant of 11 g L⁻¹ BL21-dMagR cell disruption; lane 4: bead precipitated proteins after washing of beads



Supplementary Figure S2. SDS-PAGE analysis of dMagR-his capture with magnetic beads under different conditions. (a) SDS-PAGE of purified dMagR-his diluted in respective buffers before contact with magnetic beads. 11.25 μ L of sample were applied per lane. (b) SDS-PAGE of bead precipitated proteins after washing of beads. 7.5 μ L of sample were applied per lane. dMagR-his samples in (a) and (b) were used with the following buffers: Buffers in lane 1 – 5 represent pH gradient from 5 – 12 and contained 150 mM NaCl. Lane 1: pH 7.5; Lane 2: pH 5.0; Lane 3: pH 9.0; Lane 4: pH 11.0; Lane 5: pH 12.0. Buffers in lane 6 – 9 represent samples with increasing salt at pH 7.5. Lane 6: 0 mM NaCl; Lane 7: 1000 mM NaCl; Lane 8: 2000 mM NaCl; Lane 9: 1000 mM $(\text{NH}_4)_2\text{SO}_4$.



Supplementary Figure S3. SDS-PAGE analysis for quantification of dMagR. lane 1: standard protein ladder; lane 2: supernatant of 12 g L⁻¹ BL21-Blank cell disruption lane 3: supernatant of 12 g L⁻¹ BL21-dMagR cell disruption; lane 4: 1.05 µg cytochrome c; lane 5: 2.62 µg cytochrome c; lane 6: 5.25 µg cytochrome c; lane 7: 10.50 µg cytochrome c; lane 8: standard protein ladder. The volume of the band around 14 kDa in lane 2 was subtracted from the volume of the dMagR band in lane 3 for proper quantification.