

WESTERN BLOTS

Gateway Compatible Vectors

Gateway Compatible Vectors

pcDNA3.1 (N-V5 tag)

kDa

148

98

64

50

36

22



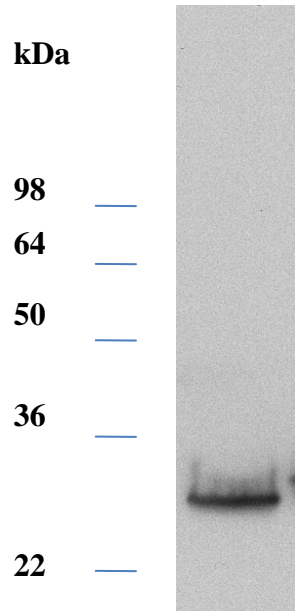
Western blot analysis confirmed the expected size of *eGFP.C3 pcDNA3.1 N-V5* protein (28kDa)

10ug of total soluble lysate (5.2ug/ul) extracted from BHK cells was loaded on a 10% polyacrylamide gel, and transferred to a nitrocellulose membrane. It was blocked with 5% dry milk in PBST overnight and probed with Mouse anti-V5 antibody at 1:2000 dilution. After washing, the membrane was incubated with horseradish peroxidase-conjugated Goat anti-mouse secondary antibody at 1:5000 dilution.

Exposure time: 5 seconds

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pcDNA-DEST40 (C-V5-6xHis tag)



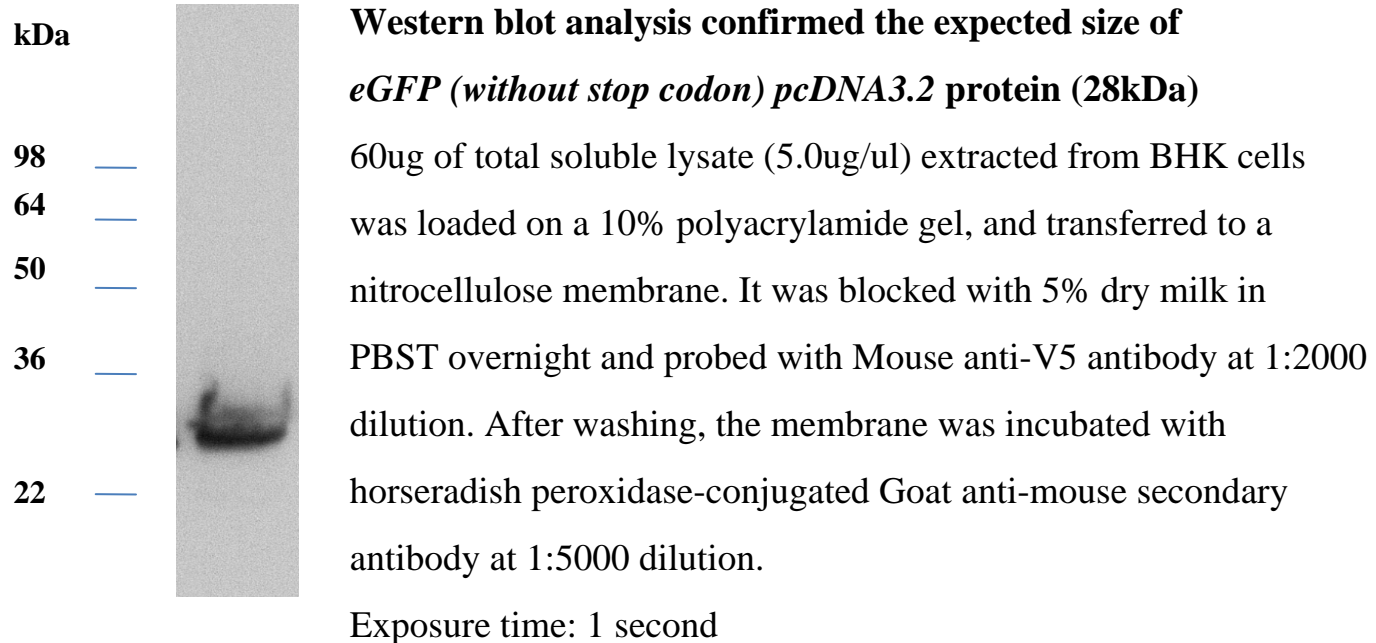
Western blot analysis confirmed the expected size of *eGFP (without stop codon) pcDNA-DEST40* protein (32kDa)

60ug of total soluble lysate (4.6ug/ul) extracted from BHK cells was loaded on a 10% polyacrylamide gel, and transferred to a nitrocellulose membrane. It was blocked with 5% dry milk in PBST overnight and probed with Mouse anti-V5 antibody at 1:2000 dilution. After washing, the membrane was incubated with horseradish peroxidase-conjugated Goat anti-mouse secondary antibody at 1:5000 dilution.

Exposure time: 1 second

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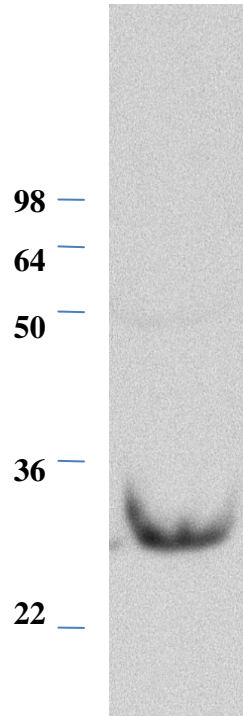
pcDNA3.2 (C-V5 tag)



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pDEST 26 (N-6xHIS tag)

kDa



Western blot analysis confirmed the expected size of *eGFP.C3 pDEST 26* protein (32kDa)

100ug of total soluble lysate (5.4ug/ul) extracted from BHK cells was loaded on a 10% polyacrylamide gel, and transferred to a nitrocellulose membrane. It was blocked with 5% dry milk in PBST overnight and probed with Mouse anti-Penta-His antibody at 1:1000 dilution. After washing, the membrane was incubated with horseradish peroxidase-conjugated Goat anti-mouse secondary antibody at 1:5000 dilution. Exposure time: 1 second

Gateway Compatible Vectors

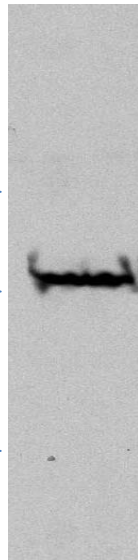
pDEST 27 (N-GST tag)

kDa

64

50

36

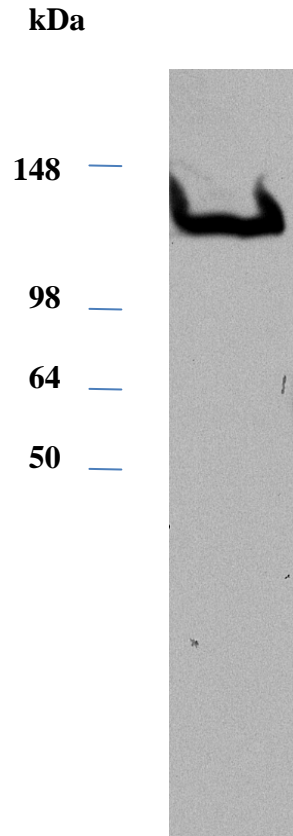


Western blot analysis confirmed the expected size of *eGFP.C3 pDEST 27* protein (53kDa)

50ug of total soluble lysate (5.1ug/ul) extracted from BHK cells was loaded on a 10% polyacrylamide gel, and transferred to a nitrocellulose membrane. It was blocked with 5% dry milk in PBST overnight and probed with Mouse anti-GST antibody at 1:2000 dilution. After washing, the membrane was incubated with horseradish peroxidase-conjugated Goat anti-mouse secondary antibody at 1:5000 dilution. Exposure time: 10 seconds

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pcDNA-DEST 53 (N-GFP tag)



Western blot analysis confirmed the expected size of *Homo sapiens ubiquitin-conjugating enzyme (FLJ13855) pcDNA-DEST 53 protein (138kDa)*

58ug of total soluble lysate (4.8ug/ul) extracted from BHK cells was loaded on a 10% polyacrylamide gel, and transferred to a nitrocellulose membrane. It was blocked with 5% dry milk in PBST overnight and probed with Rabbit anti-GFP antibody at 1:500 dilution. After washing, the membrane was incubated with horseradish peroxidase-conjugated Goat anti-rabbit secondary antibody at 1:5000 dilution.

Exposure time: 15 seconds

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pcDNA-DEST 47 (C-GFP tag)

kDa

98 —
64 —
50 —



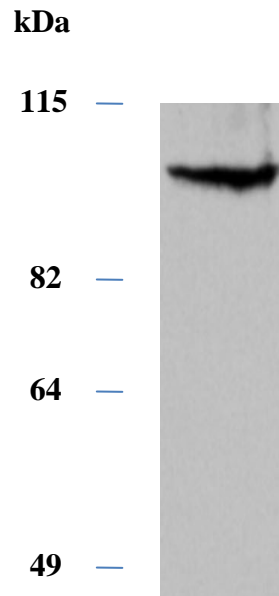
Western blot analysis confirmed the expected size of *Homo sapiens adenylsuccinate lyase (ADSL) pcDNA-DEST 47 protein (83kDa)*

70ug of total soluble lysate (4.7ug/ul) extracted from BHK cells was loaded on a 10% polyacrylamide gel, and transferred to a nitrocellulose membrane. It was blocked with 5% dry milk in PBST overnight and probed with Rabbit anti-GFP antibody at 1:500 dilution. After washing, the membrane was incubated with horseradish peroxidase-conjugated Goat anti-rabbit secondary antibody at 1:5000 dilution.

Exposure time: 15 seconds

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pcDNA6.2 N-Emerald GFP



Western blot analysis confirmed the expected size of *ATIC* pcDNA6.2 N-Emerald GFP* protein (97kDa)

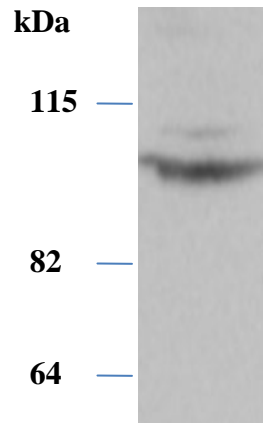
100ug of total soluble lysate (5.6ug/ul) extracted from BHK cells was loaded on a 10% polyacrylamide gel, and transferred to a nitrocellulose membrane. It was blocked with 5% dry milk in PBST overnight and probed with Rabbit anti-GFP antibody at 1:500 dilution. After washing, the membrane was incubated with horseradish peroxidase-conjugated Goat anti-rabbit secondary antibody at 1:5000 dilution.

Exposure time: 4 minutes

* *ATIC* = 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase

Gateway Compatible Vectors

pcDNA6.2 C-Emerald GFP



Western blot analysis confirmed the expected size of *ATIC* pcDNA6.2 C-Emerald GFP* protein (97kDa)

100ug of total soluble lysate (4.7ug/ul) extracted from BHK cells was loaded on a 10% polyacrylamide gel, and transferred to a nitrocellulose membrane. It was blocked with 5% dry milk in PBST overnight and probed with Rabbit anti-GFP antibody at 1:500 dilution. After washing, the membrane was incubated with horseradish peroxidase-conjugated Goat anti-rabbit secondary antibody at 1:5000 dilution.

Exposure time: 4 minutes

* *ATIC* = *5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase*

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pcDNA3.1 N-MYC

kDa

64—

50—

36—

22—



Western blot analysis confirmed the expected size of

***eGFP (without stop codon) pcDNA3.1 N-MYC* protein (27kDa)**

50ug of total soluble lysate (5.7ug/ul) extracted from

BHK cells was loaded on a 10% polyacrylamide gel, and

transferred to a nitrocellulose membrane. It was blocked with

5% dry milk in PBST overnight and probed with Mouse anti-MYC

antibody at 1:2000 dilution. After washing, the membrane was

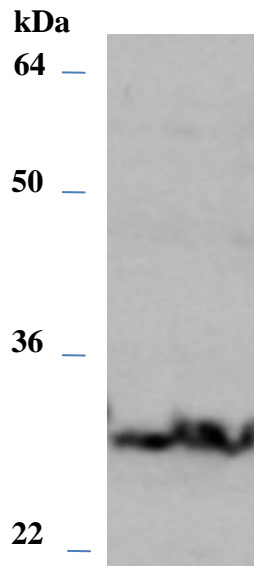
incubated with horseradish peroxidase-conjugated Goat anti-mouse

secondary antibody at 1:5000 dilution.

Exposure time: 5 minutes

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pcDNA3.2 C-MYC



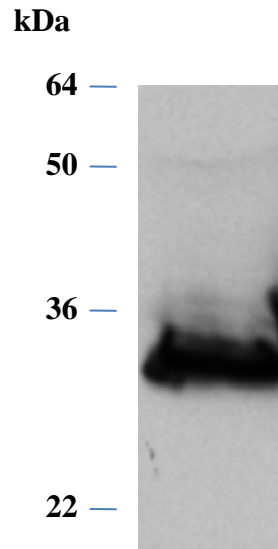
Western blot analysis confirmed the expected size of *eGFP (without stop codon) pcDNA3.2 C-MYC* protein (27kDa)

100ug of total soluble lysate (5.5ug/ul) extracted from BHK cells was loaded on a 10% polyacrylamide gel, and transferred to a nitrocellulose membrane. It was blocked with 5% dry milk in PBST overnight and probed with Mouse anti-MYC antibody at 1:5000 dilution. After washing, the membrane was incubated with horseradish peroxidase-conjugated Goat anti-mouse secondary antibody at 1:5000 dilution.

Exposure time: 5 minutes

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pcDNA3.1 N-HA

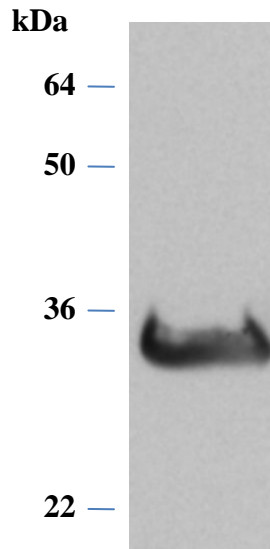


Western blot analysis confirmed the expected size of *eGFP (without stop codon) pcDNA3.1 N-HA* protein (27kDa)
50ug of total soluble lysate (5.4ug/ul) extracted from BHK cells was loaded on a 10% polyacrylamide gel, and transferred to a nitrocellulose membrane. It was blocked with 5% dry milk in PBST overnight and probed with Mouse anti-HA antibody at 1:1000 dilution. After washing, the membrane was incubated with horseradish peroxidase-conjugated Goat anti-mouse secondary antibody at 1:5000 dilution.

Exposure time: 1 second

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pcDNA3.1 N-FLAG



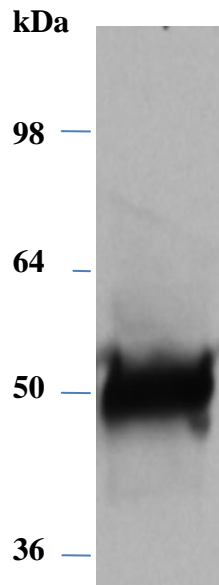
Western blot analysis confirmed the expected size of *eGFP (without stop codon) pcDNA3.1 N-FLAG* protein (27kDa)

50ug of total soluble lysate (5.5ug/ul) extracted from BHK cells was loaded on a 10% polyacrylamide gel, and transferred to a nitrocellulose membrane. It was blocked with 5% dry milk in PBST overnight and probed with Mouse anti-FLAG antibody at 1:1000 dilution. After washing, the membrane was incubated with horseradish peroxidase-conjugated Goat anti-mouse secondary antibody at 1:5000 dilution.

Exposure time: 1 second

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pDEST 15 (N-GST tag)



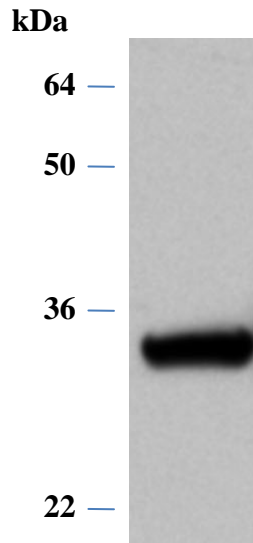
Western blot analysis confirmed the expected size of *eGFP.C3 pDEST 15* protein (54kDa)

10ug of total soluble lysate (2.7ug/ul) extracted from BL21A-I bacterial cells was loaded on a 10% polyacrylamide gel, and transferred to a nitrocellulose membrane. It was blocked with 5% dry milk in PBST overnight and probed with Mouse anti-GST antibody at 1:2000 dilution. After washing, the membrane was incubated with horseradish peroxidase-conjugated Goat anti-mouse secondary antibody at 1:5000 dilution.

Exposure time: 5 seconds

Gateway Compatible Vectors

pDEST 17 (N-6xHIS tag)

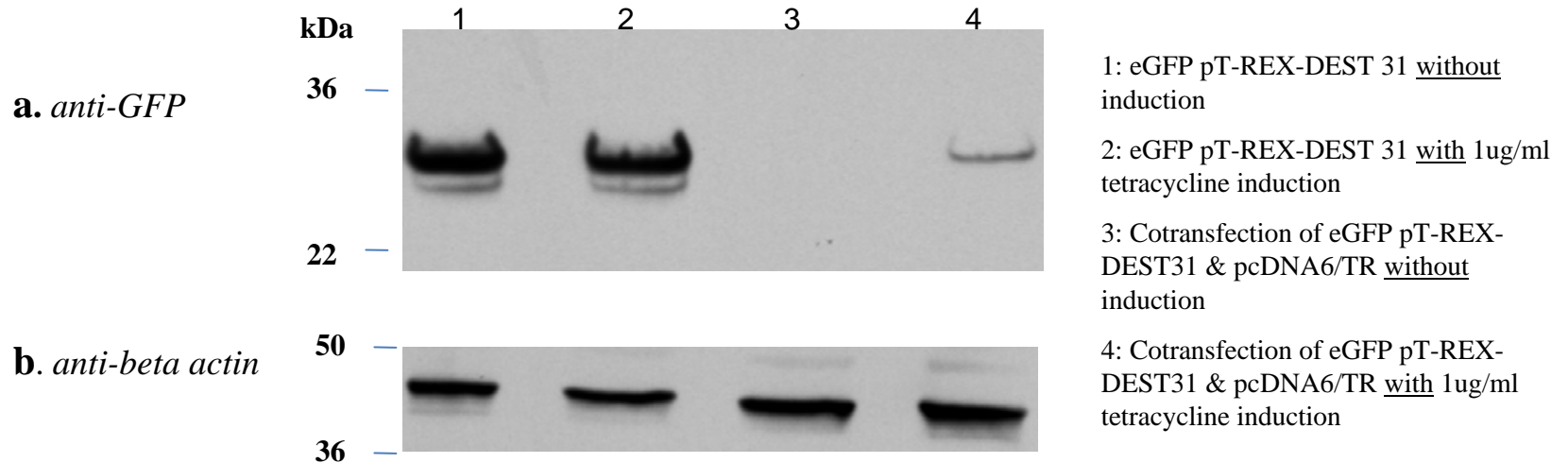


Western blot analysis confirmed the expected size of *eGFP pDEST 17* protein (32kDa)

2.5ug of total soluble lysate (5.0ug/ul) extracted from BL21A-I bacterial cells was loaded on a 10% polyacrylamide gel, and transferred to a nitrocellulose membrane. It was blocked with 5% dry milk in PBST overnight and probed with Mouse anti-Penta-HIS antibody at 1:1000 dilution. After washing, the membrane was incubated with horseradish peroxidase-conjugated Goat anti-mouse secondary antibody at 1:5000 dilution.

Exposure time: 5 seconds

Gateway Inducible Expression **pT-REX-DEST 31 & pcDNA6/TR**



eGFP pT-REX-DEST31 and pcDNA6/TR (repressor) were co-transfected at a ratio of 1:6 (w:w) into 293T cells using Calcium Phosphate transfection. Fresh medium was added 24 hours post-transfection and cells were allowed to recover for an additional 24 hours before inducing with 1ug/ml tetracycline. Cells were lysed 24 hours after induction.

- a) 50 ug of total soluble lysate was loaded on a 10% polyacrylamide gel, and transferred to a nitrocellulose membrane. It was blocked with 5% dry milk in PBST overnight and probed with Rabbit anti-GFP antibody at 1:500 dilution. After washing, the membrane was incubated with horseradish peroxidase-conjugated Goat anti-rabbit secondary antibody at 1:5000 dilution. Exposure time: 5 seconds
- b) The same membrane was stripped and probed with Mouse anti-beta actin antibody (1:5000 dilution) and horseradish peroxidase-conjugated Goat anti-Mouse secondary antibody at 1:5000 dilution. Exposure: 1 second