PRODUCT：pAID1.1-N Vector, pAID1.1-C Vector

AMOUNT：20 µg

LOT NUMBER：Specified on product label

STORAGE CONDITIONS: Store plasmid at -20 ^{o}C. Avoid repeated freeze/thaw cycles.

SHELF LIFE：One year from the date of receipt under proper storage conditions.

SHIPPING CONDITIONS：Frozen (-20 ^{o}C).

DESCRIPTION：A protein of interest fused with a 25kD-degron expressed in animal cells from pAID1.1-N Vector or pAID1.1-C Vector can be conditionally controlled by addition of auxin such as IAA, which induces rapid degradation of the target protein. Together with the target protein, the plasmids express a plant specific F-box TIR1 protein, which makes an SCF^{TIR1} complex and works as an auxin-dependent E3 ubiquitin ligase for degradation of the target protein. The plasmids can be used for transient expression as well as for establishing stable cell lines in which the protein expression of interest can be controlled by auxin.

CONCENTRATION：500 ng/µl

PLASMID SIZE：pAID1.1-N, 7438 bp; pAID1.1-C, 7450bp.

ANTIBIOTIC RESISTANCE：Kanamycin (30-50 µg/ml for E.coli), G418 (800 µg/ml for HEK293 cells)

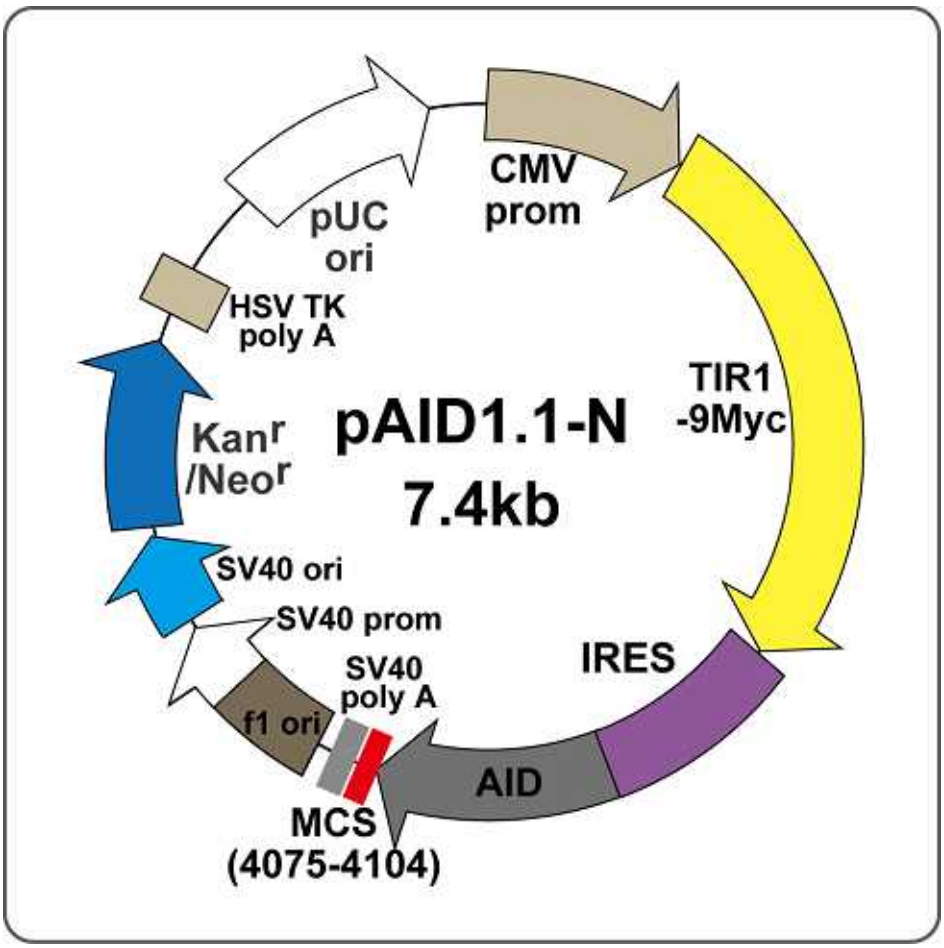
PACKAGE CONTENTS：・One tube containing either pAID1.1-N or pAID1.1-C, or alternatively two tubes containing pAID1.1-N and pAID1.1-C.

・One tube of 0.1 M indole-3-acetic acid (IAA). Store at -20 ^{o}C and avoid light.

・Vector information Packet ROIS-pAID-001

REFERENCE: Nishimura K., Fukagawa T., Takisawa H., Kakimoto T. and Kanemaki M. "An auxin-based degron system for rapid depletion of proteins in nonplant cell" *Nature Methods*, *published online 15 November 2009; DOI:10.1038/NMTH.1401*

pAID1.1-N Vector information.：



Multi Cloning Site (MCS) of pAID1.1-N

AID degron

G A G A G A G A G A

5’ GGA GCT GGT GCA GGC GCT GGA GCG GGT GCC GAT ATC GAA TTC CGA TCG ACG CGT AGT ACT 3’

Linker *EcoRV* *EcoRI* *PvuI* *MluI* *ScaI*

Restriction sites shown in MCS (EcoRV-EcoRI-PuvI-MluI-ScaI) are unique and suitable for cloning of the gene of interest.

Description：

pAID1.1-N Vector encodes both the F-box TIR1 protein and a 25-kD degron (AID degron). When cloned in frame, your protein of interest would be expressed as a fusion protein with the degron at amino (N) terminus. Both TIR1 containing 9 repeats of Myc tag (TIR1-9Myc) and the fusion genes are driven from the immediate early promoter of cytomegalovirus (CMV prom) as a bicistronic mRNA, the 3’ terminus of which is processed by the poly-adenylation signal from the SV40 T antigen. The internal ribosome binding sequence (IRES) permits translation of the aid fused protein. The

plasmid also contains an SV40 origin (SV40 ori) for replication in cells expressing the SV40 T antigen. For selection of E. coli and mammalian cells, the vector contains a kanamycin/neomycin-resistance cassette which confer resistance to kanamycin and G418, respectively. pAID1.1-N Vector also contains both a pUC origin of replication for propagation in E.coli and an f1 origin for single-stranded DNA production.

Location of Features:

- Human cytomegalovirus (CMV) immediate early promoter: 1-598
- TIR1-9Myc gene: 619-2730
- IRES sequence: 2767-3351
- AID degron: 3355-4041
- Linker: 4045-4074
- MCS: 4075-4104
- SV40 early mRNA polyadenylation signal
  Polyadenylation signal: 4256-4261 & 4285-4290; mRNA 3' ends: 4294 & 4306
- f1 single-stranded DNA origin: 4353-4808
- Bacterial promoter for expression of Kan^r gene
  -35 region: 4870-4875 ; -10 region: 4893-4898
  Transcription start site: 4905
- SV40 origin of replication: 5149-5284
- SV40 early promoter/enhancer: 4982-5211
- Kanamycin/neomycin resistance gene: 5334-6128
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal: 6363-6381
- pUC plasmid replication origin: 6412-7355

Propagation in *E. coli*:

Transform E.coli (DH5α recommended) and select them on a LB plate containing 30-50 μg/ml Kanamaycin. For preparation of plasmid, grow E.coli in liquid LB containing 30-50 μg/ml.

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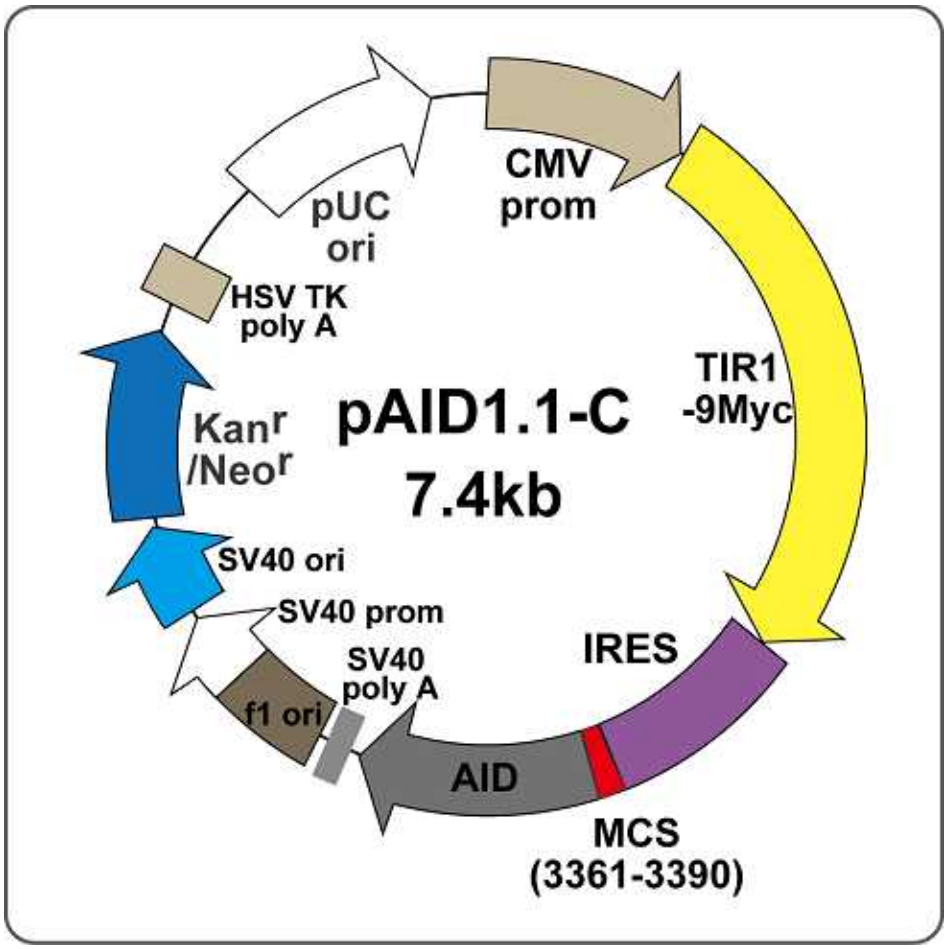
pAID1.1-C Vector information.：



Multi Cloning Site (MCS) of pAID1.1-C

Restriction sites shown in MCS (EcoRV-EcoRI-PuvI-MluI-ScaI) are unique and suitable for cloning of the gene of interest. For efficient expression of the fusion protein of interest, the gene should be in flame with the ATG codon shown above.

Description：

pAID1.1-C Vector encodes both the F-box TIR1 protein and a 25-kD degron (AID degron). When cloned in frame, your protein of interest would be expressed as a fusion protein with the degron at carboxy (C) terminus. Both TIR1 containing 9 repeats of Myc tag (TIR1-9Myc) and the fusion genes are driven from the immediate early promoter of cytomegalovirus (CMV prom) as a bicistronic mRNA, the 3' terminus of which is processed by the poly-adenylation signal from the SV40 T antigen. The internal

ribosome binding sequence (IRES) permits translation of the aid fused protein. The plasmid also contains an SV40 origin (SV40 ori) for replication in cells expressing the SV40 T antigen. For selection of E. coli and mammalian cells, the vector contains a kanamycin/neomycin-resistance cassette which confer resistance to kanamycin and G418, respectively. pAID1.1-N Vector also contains both a pUC origin of replication for propagation in E.coli and an f1 origin for single-stranded DNA production.

Location of Features:

- Human cytomegalovirus (CMV) immediate early promoter: 1-598
- TIR1-9Myc gene: 619-2730
- IRES 2767-3351
- MCS: 3361-3390
- Linker: 3391-3420
- AID degron: 3430-4116
- SV40 early mRNA polyadenylation signal
  Polyadenylation signal: 4268-4273 & 4297-4302; mRNA 3' ends: 4306 & 4318
- f1 single-stranded DNA origin: 4365-4820
- Bacterial promoter for expression of Kan^r gene
  -35 region: 4882-4887; -10 region: 4905-4910
  Transcription start site: 4917
- SV40 origin of replication: 5161-5296
- SV40 early promoter/enhancer: 4994-5223
- Kanamycin/neomycin resistance gene: 5346-6140
- Herpes simplex virus (HSV) thymidine kinase (TK) polyadenylation signal: 6375-6393
- pUC plasmid replication origin: 6724-7367

Propagation in *E. coli*:

Transform E.coli (DH5a recommended) and select them on a LB plate containing 30-50 μg/ml Kanamaycin. For preparation of plasmid, grow E.coli in liquid LB containing 30-50 μg/ml.

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